USER GUIDE

Pharmacogenomics Experiments

For use with:

TaqMan® OpenArray® Genotyping Plates, QuantStudio® 12K Flex System

QuantStudio® 12K Flex System with OpenArray® Block (with Accufill™ System)

TaqMan® Genotyper™ Software

CopyCaller® Software

AlleleTyper™ Software

Catalog Numbers 4475395, 4471090, and 4486060

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Revision B.0

For Research Use Only. Not for use in diagnostic procedures.

Contents

About this guide

IMPORTANT! Before using products described in this manual, read and understand the information in the "Safety" appendix in this document.

Revision history

Revision	Date	Description of change
B.0	4 August 2014	• DNA sample preparation procedures in Chapter 4 updated for use of the MagMAX™ DNA Multi-Sample Ultra Kit. Users referred to a new Appendix A for complete procedures. • "Perform analysis in TaqMan® Genotyper™ Software" topic in Chapter 5 reorganized for better workflow. • Sample quantification procedure using the RNase P Detection Reagents Kit added to a new Appendix B. • Corrections and clarifications made to Appendix C Troubleshooting.
A.0	21 January 2014	New document

Purpose

The Pharmacogenomics Experiments User Guide provides procedures for using Life Technologies products to perform flexible, scalable, fast, and economical pharmacogenomic (PGx) experiments.

1 About the PGx Workflow

This chapter covers:

Introduction

Pharmacogenomics (PGx) is the study of genetic variation as it relates to drug response. PGx studies involve testing samples for multiple variants in drug metabolism enzyme (DME) and transporter genes. The *Pharmacogenomics Experiments User Guide* functions as a complete guide for Life Technologies' complete sample-to-result PGx workflow solution using the QuantStudio® 12K Flex Real-Time PCR System.

Workflow

1. Select TaqMan® SNP/DME Genotyping Assays 2. Order OpenArray® plates	1. Select TaqMan® Copy Number Assays 2. Order single tube assays

Purify and quantify gDNA samples

(Optional) Split samples and perform preamplification for OpenArray® plates

Run SNP genotyping experiments on OpenArray® plates • Review data in QuantStudio® 12K Flex Software • Review data in TaqMan® Genotyper™ Software • Export TaqMan® Genotyper™ Software results file	Run copy number experiments on 96- or 384-well plates • Review data in QuantStudio® 12K Flex Software • Review data in CopyCaller® Software • Export CopyCaller® Software results file

Run translational analysis in AlleleTyper™ Software

Note: See Appendix D for system specifications.

2 Background and tools for assay content selection

This chapter covers:

About assay content selection

Pharmacogenomic studies involve testing samples for multiple variants in drug metabolism enzyme (DME) and transporter genes. Life Technologies offers a comprehensive collection of validated TaqMan® Drug Metabolism Genotyping Assays that are optimized for genotyping single nucleotide polymorphisms (SNPs), insertions and deletions (InDels), and multi-nucleotide polymorphisms (MNPs) in drug metabolism-related genes in the research setting. The DME Assays collection of 2,700 assays detect potentially causative polymorphisms in 221 drug metabolism enzyme (DME) and associated transporter genes. As well, 4.5 million predesigned SNP assays and custom SNP assays are available for other targets of interest. In addition, TaqMan® Copy Number Assays are available for examining copy number variation (CNV) in DME genes.

This chapter covers an introduction to DME gene allele nomenclature, TaqMan® SNP and DME Genotyping Assays, TaqMan® Copy Number Assays, and tools and information required for finding and selecting assays for your study.

Allele nomenclature

PGx researchers familiar with drug metabolism genes frequently know a particular SNP by its standardized allele name or “star (*) allele nomenclature” (Sim et al. 2010). Star alleles are gene-level haplotypes (a set of DNA polymorphisms that tend to be inherited together on the same chromosome). In many cases, these haplotypes have been associated with DME activity levels (e.g. functional, decreased function, or nonfunctional variants). The combination of star allele haplotypes (i.e., the diplotype) within a sample can be used to predict the DME phenotype (e.g., ultrarapid, extensive, intermediate, or poor). Genetic variants within a haplotype can include SNPs, InDels, and CNVs. The allele nomenclature for a specific gene family is maintained and standardized by an affiliated group of scientists that curate each site independently. This nomenclature can be complicated, because many alleles contain more than one polymorphism (i.e., they are haplotypes) and conversely, many polymorphisms are associated with several alleles.

The star allele nomenclature contains the DME gene name, such as CYP2D6, followed by a numeric allele name, such as *3. A star allele conventionally contains at least one causative variant (e.g., a frameshift mutation). Variants are given reference gene and/or cDNA coordinates, such as g.2549delA (full variant name: CYP2D6*3 g.2549delA). The causative star allele variant may be associated with other nucleotide variants in different haplotypes groups; such sub-alleles are denoted by letters following the numeric allele identifier (e.g., *3A). On the Cytochrome P450 (CYP) Allele Nomenclature web site, the defining, causative variant for a star allele is often in bold font.

Note: *1 refers to the reference gene sequence, which has normal function. The reference gene sequence is not necessarily equivalent to the reference genome assembly sequence and it does not necessarily contain the major allele for a given SNP (which can vary between populations, particularly for highly polymorphic SNPs).

The defining variant for a given DME gene star allele may be the only variant that needs to be interrogated to identify that particular star allele. The defining allele is sometimes referred to as the "common allele". Common allele names are provided for many DME variants in the PGx Common Markers file (see "Select assays" on page 15).

The DME Assay collection variants have been mapped for the genes having public allele nomenclature sites. This allele nomenclature is searchable on the DME assay product pages and in the downloadable TaqMan® Drug Metabolism Genotyping Assays Index file, available on the Life Technologies website. The public allele nomenclature websites provide information on known DME gene star allele haplotypes, the defining polymorphisms for these alleles, and in many cases, links to the NCBI dbSNP website if an rsSNP identifier has been assigned.

Table 1 DME allele nomenclature websites

Gene family	Allele nomenclature website
CYP - Cytochrome P450 (CYP) genes	http://www.cypalleles.ki.se
NAT1 and NAT2 - Arylamine N-Acetyltransferase genes	http://nat.mbg.duth.gr
UGT - UDP Glucuronosyltransferase genes	http://www.pharmacogenomics.pha.ulaval.ca/cms/ugt_alleles

Note: Other DME gene variants/alleles have nomenclature that is reported in the literature, but there is no public nomenclature web site for them. For some key variants, allele nomenclature can be found in the Very Important PGx (VIP) gene summary pages on the Pharmacogenomics Knowledge Base web site **http://www.pharmgkb.org**.

Where applicable and possible, such allele nomenclature has been provided for non-CYP, -NAT and -UGT variants in the PGx Common Markers file (see "Select assays").

Select assays

TaqMan® SNP/ DME Assays

The TaqMan® Drug Metabolism Genotyping Assays, a collection of 2,700 assays, detect potentially causative SNP, MNP or InDel polymorphisms in 221 drug metabolism enzyme (DME) and associated transporter genes. The DME assays were designed using an optimized DME assay design algorithm that uses a high level of bioinformatics to ensure a lack of underlying polymorphisms and high target specificity (i.e. gene family members and pseudogenes will not amplify). Manual design was required for some of the more difficult targets. All DME Assays underwent stringent wet-lab validation, including testing with 180 unique DNA samples from four different populations (African American, Caucasian, Chinese and Japanese). The 4.5 million TaqMan® SNP Genotyping Assays were designed using a related, highly validated SNP assay design algorithm. The SNP assays are functionally tested (i.e. for amplification and clustering capabilities) when first manufactured on 20 unrelated gDNA samples from three populations (African American, Caucasian, and Japanese). In addition, over 300 high value TaqMan® DME and SNP assays were tested with 44 African American and Caucasian samples and synthetic templates representing each genotype, on OpenArray® plates run on the QuantStudio® 12K Flex Real-Time PCR System, to ensure assay performance on this platform.

All TaqMan® SNP Genotyping Assays, Drug Metabolism Genotyping Assays, and Custom SNP Genotyping Assays contain sequence-specific forward and reverse primers to amplify the polymorphic sequence of interest and two TaqMan® MGB probes with Non-fluorescent quencher (NFQ):

- One probe labeled with VIC® dye detects the Allele 1 sequence.
- One probe labeled with FAM™ dye detects the Allele 2 sequence.

TaqMan® Drug Metabolism and SNP Genotyping Assays may be ordered as single tube assays or pre-plated on OpenArray® plates (see Chapter 3, “Ordering information” on page 27).

TaqMan® DME and SNP Genotyping Assay data is analyzed by cluster plot analysis: FAM™ dye signal is plotted on the Y-axis and VIC® dye signal is plotted on the X-axis. Samples homozygous for the FAM™- or VIC®-labeled alleles will form clusters along the Y- or X-axis, respectively, whereas heterozygous samples contain both FAM™ and VIC® dye signal and will cluster roughly along the diagonal position between the homozygous clusters.

Genotyping assay context sequences

The reporter dye information for TaqMan® Drug Metabolism and SNP Genotyping Assays is represented in the assay context sequence, which is posted on the Life Technologies web site and provided in the Assay Information File that you can download from the web at **www.lifetechnologies.com/OA-platefiles**. The context sequence is the nucleotide sequence surrounding the SNP site. It is provided in the (+) genome strand orientation relative to the NCBI reference genome. The SNP alleles are included in brackets, where the order of the alleles corresponds to the association with probe reporter dyes, where [Allele 1 = VIC® dye / Allele 2 = FAM™ dye].

For example, the C__27102431_D0 assay, which targets the CYP2D6*4 g.1846G>A SNP, rs3892097, has the following context sequence:

```
AGACCGTTGGGGCGAAAGGGGCGTC[C/T]TGGGGGTGGGAGATGCGGGTAAGGG
```

The VIC® dye probe is associated with the C allele and the FAM™ dye probe is associated with the T allele.

Note that, in this example, the SNP alleles (C/T) are the reverse complement of those given in the star allele nomenclature: CYP2D6*4 g.1846G>A. This is because the context sequence alleles are provided in the (+) reference genome strand orientation whereas the star allele nucleotide changes are provided with respect to the CYP2D6 gene reference sequence that maps to the (-) genome strand.

TaqMan® Copy Number Assays

Copy number variation must be assessed for DME genes that are known to exhibit copy number variation (see “DME genes and copy number variation” on page 20). TaqMan® Copy Number Assays are run simultaneously with a TaqMan® Copy Number Reference Assay in a duplex real-time polymerase chain reaction (PCR). The Copy Number Assay detects the target gene or genomic sequence of interest and the Reference Assay detects a sequence that is known to exist in two copies in a diploid genome (e.g., the human RNase P H1 RNA gene). The number of copies of the target sequence in each test sample is determined by relative quantitation (RQ) using the comparative C_T ($\Delta\Delta C_T$) method.

- TaqMan® Copy Number Assay contains two primers and a FAM™ dye-labeled MGB probe to detect the genomic DNA target sequence.
- TaqMan® Copy Number Reference Assay contains two primers and a VIC® dye-labeled TAMRA™ probe to detect the genomic DNA reference sequence.

TaqMan® Copy Number Assays can be ordered as single tube assays (see Chapter 3, “Ordering information” on page 27) and run on 96-well and 384-well plates on Applied Biosystems Real Time instruments, including the QuantStudio® 12K Flex Real-Time PCR System. A copy number assay protocol has not yet been developed for the OpenArray® plate.

Search using the Assay Search Tool

About the Life Technologies website Assay Search Tool

The Life Technologies web site provides an easy-to-use assay search tool to aid selection of TaqMan® DME, SNP and Copy Number Assays. The search tool can be accessed from the Real-Time PCR Assays page or any of the specific product pages accessed from the Life Technologies web site.

Search for TaqMan® SNP Genotyping assays

To search for TaqMan® DME or SNP Genotyping Assays using the Assay Search tool, select the **SNP Genotyping** experiment type. You can choose to search for All SNP Genotyping/Human (includes the DME Assays) or just the validated Drug Metabolism Assays collection. Search terms include gene symbol, rsSNP ID, allele nomenclature, and assay ID. Single keyword or batch search options are available, as is a search by genomic location option.

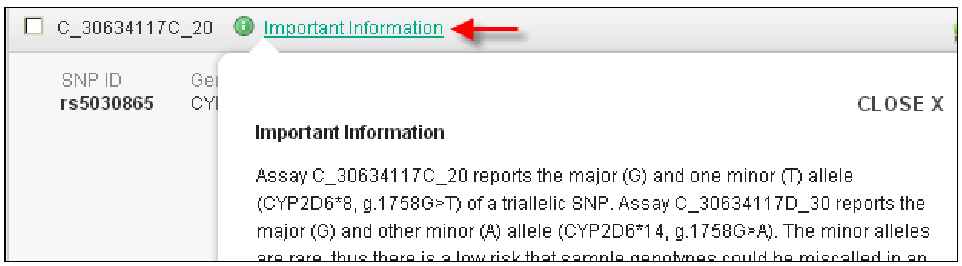
- **View assay search result.** The search results page will include the following information for each assay returned:
  - Assay ID
  - SNP ID (NCBI dbSNP rs ID or human Celera Variant hCV ID if no rs ID is available)
  - Gene
  - Location (genomic coordinate: chr.#:coordinate)

– SNP Type (e.g. missense mutation)
– Assay type (e.g. DME)

If many assays are returned in a search, the option to filter results will be given in the left side bar (e.g. filter by gene, assay type, SNP type, etc.).

Note: If allele nomenclature is used as a search term (e.g. CYP2D6*4), all DME assays to variants within all associated sub-alleles will be returned. If needed, review the variants on the associated allele nomenclature website to determine which are important to evaluate in your study (e.g. only the allele-defining variants may be of interest).

IMPORTANT! Read any Important Information notes associated with the assay (click the **Important Information** box that appears beside that Assay ID on the assay search results bar to open a pop-up box). These provide information required for making ordering decisions. For example, a copy number assay may be required in addition to the DME assay for sample genetic analysis, or the DME assay may be one of a pair of assays interrogating a triallelic SNP.



- **View Details.** Additional annotations can be viewed for any assay by clicking the **Assay Details** button. Additional annotations include:
  – SNP ID - links to NCBI dbSNP page
  – Context Sequence - provided in the (+) reference genome strand orientation; the bracketed SNP alleles denote the dye/allele association ([VIC®/FAM™])
  – Nomenclature (if applicable) - links to CYP, NAT and UGT allele nomenclature web sites
  – Gene details (transcript accession, SNP location, SNP type, codon change, amino acid change)
- **View allele frequency information.** If minor allele frequency (MAF) information is available for a SNP, click the **Allele Frequency** button to view MAF information generated by Applied Biosystems (AB), HapMap, or Applera Genome Initiative (AGI).
- **View full product details.** To view an assay page containing all information for a given assay, click the **View Full Product Details** button at the bottom of the Assay Details page.
- **View Assay on Map.** Click the **View Assay on Map** link to view the selected assay in its genomic context along with other neighboring assays.

- **Add to Cart.** To order single tube assays, click **Add to Cart**. To order a panel of assays for an OpenArray® plate, export a list of assays for import into the OpenArray® Configurator (see Chapter 3, "Ordering information" on page 27).
- **Export search results.** The returned assay list can be exported to look over before ordering, and to provide a list of assays for an OpenArray® order. The export file will contain the assay ID along with annotations including catalog number, SNP, gene, context sequence, genomic location, allele nomenclature, and MAF (AB, HapMap, AGI) annotations.

Search for TaqMan® Copy Number Assays

To search for TaqMan® Copy Number Assays using the Assay Search tool, select the **Copy Number** experiment type and the **human** species. Search terms include gene symbol, assay ID, and Database of Genomic Variants (DGV) variation IDs. Single keyword or batch search options are available, as is a search by genomic location.

- **View assay search result.** The assay search result page will display the assay ID along with the genomic location coordinate (position within the assay probe sequence), the gene symbol, number of associated Database of Genomics Variants (DGV) variant IDs, cytoband, and amplicon length. Pre-tested TaqMan® Copy Number assays are available for several DME genes known to lie with copy number variant regions (see "DME genes and copy number variation" on page 20). These can be identified by searching by gene symbol or name and then applying the "pre-tested" assay filter to the results. A "Pre-tested" note will appear in a box beside the assay ID in the search results, indicating that the assay was run on 90 samples: 45 each from African American and Caucasian populations.

IMPORTANT! Read any "Important Information" notes associated with the Assay ID in the search results. These provide information required for making ordering decisions, for example, information on the particular star alleles that an assay detects.

- **Required reference assays.** Copy Number Assays must be run in duplex PCR with TaqMan® Copy Number Reference Assay: RNase P (default assay) and TERT (alternate assay) assays are available, and you can add them to your shopping cart via the Required Reference Assays box.
- **View assay details.** Additional annotations can be viewed for any assay by clicking the **Assay Details** button. Additional annotations include:
  - Target Gene details (e.g. assay gene and transcript locations) – links to NCBI
  - Target Copy Number Variation Details (e.g. copy number variants that the copy number assay maps to) – links to DGV
- **View full product details.** To view an assay page containing all information for a given assay, click the **View Full Product Details** button at the bottom of the Assay Details page.
- **View assay on map.** Click the **View Assay on Map** link to view the selected assay in its genomic context along with other neighboring assays.

- **Add to cart.** To order single tube assays, click **Add to Cart**. Remember to add the required copy number reference assay along with your target copy number assays.
- **Export search results.** You can export the returned assay list to look over before ordering. The export file will contain the assay ID along with annotations including catalog number, gene, DGV and genomic location annotations.

Search the TaqMan® Drug Metabolism Genotyping Assays Index

The TaqMan® Drug Metabolism Genotyping Assays Index can be downloaded from **www.lifetechnologies.com/taqmandme.** This file contains a comprehensive list of the DME assays along with annotations listed below. This file can facilitate looking for DME assays to polymorphisms of interest given the extensive annotation information within it, which includes:

- Gene symbol and name
- NCBI SNP reference (if applicable)
- Polymorphism (e.g., A/G)
- Amino acid change (if applicable)
- Allele nomenclature (if available)
- Polymorphism (for example, A/G)
- SNP type (e.g. missense mutation)
- Context sequence [VIC®/FAM™] (in plus strand orientation with SNP alleles in brackets)
- Applied Biosystems minor allele frequency data (Caucasian, African American, Japanese, Chinese populations)

Search the PharmaADME Core Marker Set

The PharmaADME consortium (**www.PharmaADME.org**), composed of individuals from academia, pharmaceutical, and genomic technology industries, created a consensus list of known and putative functional variants in key genes involved in the absorption, distribution, metabolism, and excretion (ADME) of drugs. A Core Marker list of variants considered most likely to impact drug metabolism was composed of 184 variants in 33 key ADME genes.

Life Technologies developed TaqMan® assays to the PharmaADME Core Markers (> 95% coverage). This assay set is comprised of both TaqMan® DME and Copy Number assays to 172 SNP, InDel, and CNV targets:

- 164 DME assays include 10 assays to genotype 5 triallelic SNPs (paired assays)
- 10 copy number assays cover deletions and duplications in 6 total DME genes

A tabular list of the available assays can be downloaded at:

http://tools.lifetechnologies.com/content/sfs/brochures/cms_082106.xls

Search the PGx Common Markers file

Life Technologies has pretested over 300 TaqMan® DME and SNP assays to highly studied, important DME and other gene variants on OpenArray® plates run on the QuantStudio® 12K Flex Real-Time PCR System. All assays on this list were tested on this system with 44 Coriell gDNAs (22 each African American and Caucasian samples, a subset of the DME Assay 90 sample validation panel) and most were also tested with synthetic plasmid constructs representing each genotype. You can download the PGx Common Markers file that contains a list of the most commonly requested assays from this set at:

www.lifetechnologies.com/pgx

The file includes common allele names, context sequences, and other useful annotations.

Note: From the same web page you can also download *TaqMan DME Genotyping Assays on OpenArray Plates*, a presentation containing screen shots of the test data for the most commonly requested PGx marker assays.

Special assay considerations

DME genes and copy number variation

Several DME genes exhibit Copy Number Variation (CNV) or other structural alterations due to recombination and gene conversions events between highly related loci (He et al. 2011). Pre-tested TaqMan® Copy Number assays are available for these genes. These assays were run on 90 Coriell gDNA samples from African American and Caucasian populations (the same panel used for DME Assay validation). The pretested assays for the DME genes in CNV regions are listed in on page 20, along with their target location in the gene and the major DME alleles that can be tested by these assays.

Table 2 Pre-tested DME gene TaqMan® Copy Number Assays for CNV and hybrid gene analysis

Gene symbol	Assay ID	Gene location	Major alleles tested
CYP2D6	**Hs04083572_cn**	Intron 2	CYP2D6 Deletion (*5); Duplications (*1xN, *2xN, *4x2, *9x2,*10x2, *17xN, *35x2); 2D6/2D7 hybrid alleles with 2D7 exon 9 sequences (*36, *83)
CYP2D6	Hs04502391_cn	Intron 6	CYP2D6 Deletion (*5); Duplications (*1xN, *2xN, *4x2, *9x2,*10x2, *17xN, *35x2); 2D6/2D7 hybrid alleles with 2D7 exon 9 sequences (*36, *83)
CYP2D6	**Hs00010001_cn**	Exon 9	CYP2D6 Deletion (*5); Duplications (*1xN, *2xN, *4x2, *9x2,*10x2, *17xN, *35x2)
CYP2A6	Hs07545273_cn	Exon 1	CYP2A6 Deletion (*4); Duplication (*1x2)
CYP2A6	**Hs07545274_cn**	Intron 1	CYP2A6 Deletion (*4); Duplication (*1x2)
CYP2A6	Hs04488984_cn	Intron 2	CYP2A6 Deletion (*4); Duplication (*1x2); hybrid allele with 2A7 exons 1-2 and 2A6 exons 3-9 (*12)
CYP2A6	**Hs07545275_cn**	Intron 7	CYP2A6 Deletion (*4); Duplication (*1x2); hybrid allele with 2A7 exons 1-2 and 2A6 exons 3-9 (*12)
CYP2A7	**Hs07545276_cn**	Exon 1	CYP2A6 hybrid allele with 2A7 exons 1-2 and 2A6 exons 3-9 (*12)
CYP2A7	Hs04488016_cn	Intron 2	CYP2A6 hybrid allele with 2A7 exons 1-2 and 2A6 exons 3-9 (*12)
CYP2A7	**Hs07545277_cn**	Intron 7	No CYP2A6 alleles
CYP2E1	Hs00010003_cn	Promoter	CYP2E1 Duplication (*1x2)
GSTM1	Hs02575461_cn	Exon 1	GSTM1 Deletion (*0); Duplication
GSTT1	Hs00010004_cn	Intron 1	GSTT1 Deletion (*0)
SULT1A1	Hs03939601_cn	Intron 2	SULT1A1 Deletion; Duplication
UGT2B17	Hs03185327_cn	Exon 1	UGT2B17 in 150 kb Deletion (*2)

Note: For the CYP genes having multiple assays available, those assays in boldface type are the most frequently used for CNV and hybrid gene analysis.

Note: The copy number assays are not SNP allele-specific and cannot be used to determine which SNP allele is duplicated when a sample is heterozygous and has three gene copies.

For DME gene variants that are associated with copy number variation, it is necessary to run both DME genotyping assays and copy number assays to determine sample genotypes. For a given DME assay that targets a gene that can be deleted or duplicated in individuals (e.g. CYP2D6), samples having no gene will not amplify, samples having 1 or more copies that are homozygous for the SNP allele will cluster together, and samples having more than 2 copies that are heterozygous may run within the 2-copy heterozygous cluster or between it and one of the homozygous clusters.

Copy number quantitation of the gene target must thus be used to determine which samples carry gene deletions or duplications to discern the sample genotype. Additionally, use of multiple copy number assays for a gene may be required for hybrid gene analysis.

Note that the copy number assays are not SNP allele-specific and cannot be used to determine which SNP allele is duplicated when a sample is heterozygous and has three gene copies.

GSTM1 and GSTT1 DME assays and CNV

The GSTM1 and GSTT1 genes have a very high frequency of deletion and are entirely missing in a substantial number of individuals in multiple populations. This means that DME genotyping assays to variants within these genes will usually yield results wherein many samples do not amplify and cluster with the no template controls (NTCs).

CYP2D6 copy number variation and CYP2D6/CYP2D7 hybrid alleles

The CYP2D6 gene is the most highly polymorphic and complex of the DME genes. It is also of primary importance as it is responsible for the metabolism of about 25% of current drugs. Over 100 star allele groups have been identified by the Cytochrome P450 Nomenclature Committee (**http://www.cypalleles.ki.se**). At least 4 of these groups give rise to alleles with substrate-dependent reduced enzyme activity, and more than 20 do not encode functional enzymes. The CYP2D6 alleles are composed of SNP and InDel variants, CNVs, and hybrid alleles formed by recombination between CYP2D6 and highly related upstream pseudogene, CYP2D7, sequences. Individuals may carry null alleles (*5) or extra copies of CYP2D6 (*1, *2, *4, *9,*10, *17, *35). Some CYP2D6 alleles contain sequences derived from the highly homologous CYP2D7 pseudogene; e.g. CYP2D6*36, as well as *4N and *83, contains a gene conversion to CYP2D7 sequences in exon 9 associated with negligible CYP2D6 enzyme activity and poor metabolizer phenotype (Gaedigk et al. 2006).

Three different copy number assays to CYP2D6 sequences are available for determining CYP2D6 gene copy number and to aid identification of some hybrid alleles (see "DME genes and copy number variation" on page 20):

- Hs00010001_cn specifically targets CYP2D6 exon 9 sequences, and it will not amplify CYP2D7 or CYP2D8 pseudogenes or CYP2D6/CYP2D7 hybrid alleles carrying CYP2D7 exon 9 sequences (e.g. CYP2D6*36).
- Hs04083572_cn specifically targets CYP2D6 intron 2 sequences and Hs04502391_cn specifically targets CYP2D6 intron 6 sequences. These assays will not amplify pseudogenes, but they will amplify CYP2D6/CYP2D7 hybrid alleles carrying CYP2D6 intron 2 and intron 6 sequences, respectively (e.g. CYP2D6*36).

The primary CYP2D6 copy number assay for CNV analysis is the exon 9 Hs00010001_cn assay that predominantly detects full-length CYP2D6 alleles and not the fairly common, non-functional *36 allele. If copy number information for both CYP2D6 and hybrid genes, including the CYP2D6*36 allele, is desired, then the intron 2 and/or intron 6 assays can additionally be used (Ramamoorthy et al. 2010). In some cases, adding intron 2 and/or intron 6 assays is required for proper sample genotyping. For example, use of upstream intron 2 and/or intron 6 assays along with the exon 9 assay can help to identify *13 alleles that carry upstream CYP2D7 sequences and downstream CYP2D6 sequences; if the exon 9 assay alone is used, the *13 non-functional alleles will go undetected and be counted as normal *1 alleles.

CYP2A6 CNV and CYP2A6/CYP2A7 hybrid alleles

Currently, 38 CYP2A6 alleles have been defined by the Cytochrome P450 Nomenclature Committee (**http://www.cypalleles.ki.se**). At least 4 of these alleles do not encode functional enzymes and several others encode reduced function enzymes. The genotyping of CYP2A6 alleles is complicated by the presence of copy number variant deletion (*4) and duplication (*1) alleles, and hybrid alleles formed by recombination with upstream pseudogene CYP2A7 sequences, e.g. the reduced function *12 allele contains exons 1-2 of CYP2A7 origin and exons 3-9 of CYP2A6 origin (Oscarson et al. 2002).

Several copy number assays to CYP2A6 and CYP2A7 sequences are available for examining copy number variants and hybrid alleles (see "DME genes and copy number variation" on page 20 for the complete list).

- Hs07545274_cn specifically targets CYP2A6 intron 1 sequences and Hs07545273_cn specifically targets 5′ end CYP2A6 sequences within the promoter and exon 1. These assays will not amplify CYP2A7 or CYP2A6/CYP2A7 hybrid alleles carrying CYP2A7 intron 1 or 5′ end sequences, respectively (e.g. CYP2A6*12).
- Hs07545275_cn specifically targets CYP2A6 intron 7 sequences and Hs04488984_cn specifically targets CYP2A6 intron 2 sequences. These assays will not amplify CYP2A7, but they will amplify CYP2A6/CYP2A7 hybrid alleles carrying CYP2A6 intron 7 or intron 2 sequences, respectively (e.g. CYP2A6*12).
- Hs07545276_cn specifically targets CYP2A7 exon 1 sequences, and it will not amplify CYP2A6 but will amplify CYP2A6/CYP2A7 hybrid alleles carrying CYP2A7 exon 1 sequences (e.g. CYP2A6*12).
- Hs07545277_cn specifically targets CYP2A7 intron 7 sequences and Hs04488016_cn specifically targets CYP2A7 intron 2 sequences. These assays will not amplify CYP2A6 or CYP2A6/CYP2A7 hybrid alleles carrying CYP2A6 intron 7 or introns 2 sequences, respectively (e.g. CYP2A6*12).

At least two assays are required to detect copy number variation in CYP2A6. The CYP2A6 intron 7 assay can be used to detect *4 deletion and *1 duplication alleles. This assay will amplify both full length CYP2A6 and the partially active CYP2A6/CYP2A7 hybrid allele, CYP2A6*12, but will not be able to discern them. The intron 1 assay can also be used to detect *4 deletion and *1 duplication alleles. This assay will amplify full length CYP2A6 but will not be able to amplify the CYP2A6*12 hybrid allele. If both CYP2A6 assays are run, *12 alleles can be distinguished from full-length alleles. For example, a *1/*12 sample will give 2 copies using the intron 7 assay and 1 copy using the intron 1 assay, whereas a *1/*1 sample will give 2 copies with both assays.

Additionally, CYP2A7 assays can be used to corroborate the presence of CYP2A6*12 alleles: The CYP2A7 exon 1 assay will amplify the hybrid CYP2A6*12 allele, whereas the intron 7 assay will not amplify intact CYP2A6 or the CYP2A6*12 allele.

TaqMan® DME Assays for genotyping triallelic SNPs

Several important DME gene variants are triallelic SNPs wherein 3 bases occur at the same genomic location. Triallelic SNP targets can be interrogated using a pair of TaqMan® assays. Each assay contains one probe for the major SNP allele, which is labeled with the same reporter dye (VIC® dye) in both assays, and one probe for one of the minor alleles, which is labeled with the second reporter dye (FAM™ dye). To generate accurate sample genotypes, the two assays must be run independently on the same panel of samples, and the resulting allelic discrimination plots must be analyzed in concert, comparing the expected cluster positions from both assays to a map of the true sample genotypes. Sample genotypes can be assigned with the help of a spreadsheet program and a chart of the expected genotypes (see Table 10 on page 65) or the AlleleTyper™ Software can be used to translate the results.

See the application note *TaqMan® Drug Metabolism Genotyping Assays for Triallelic SNPs* (Pub. no. 135AP01-01) for more details on manual analysis of triallelic SNPs using 2 DME SNP assays or refer to the *AlleleTyper Software User Guide* (Pub. no. 4469874) for information on having the genotypes assigned automatically by this software.

Table 3 TaqMan® DME Assays to triallelic SNPs

Gene	rsSNP ID	TaqMan® DME assay	Allele name	Assay alleles
ABCB1	rs2032582	C_11711720C_30	ABCB1 c.3095G>T	C/A
		C_11711720D_40	ABCB1 c.3095G>A	C/T
CYP2C9	rs7900194	C__25625804_10	CYP2C9*8 c.449G>A	A/G
		C_25625804D_20	CYP2C9*27 c.449G>T	T/G
CYP2D6	rs5030865	C_30634117C_K0	CYP2D6*8 g.1758G>T	A/C
		C_30634117D_M0	CYP2D6*14 g.1758G>A	T/C
CYP1A1	rs41279188	C_30634152C_70	CYP1A1*5 g.2461C>A	G/T
		C_30634152D_80	CYP1A1*9 g.2461C>T	G/A
CYP2C8	rs72558195	C_72650009C_10	CYP2C8*7 c.556C>T	G/A
		C_72650009D_20	CYP2C8*8 c.556C>G	G/C

Note: On the Life Technologies Assay Search results pages, the annotation for the DME assays is tied to the SNP ID and not the SNP alleles. Therefore, both assays for a triallelic SNP will be assigned the same allele nomenclature. Refer to the Important Information and/or the context sequence to determine which alleles are interrogated by each assay.

Example: ABCB1 c.3095G>T/A triallelic SNP rs2032582 assays

```
C_11711720C_30 ABCB1c.3095 G>T
TATTTAGTTTGACTCACCTTCCCAG[C/A]ACCTTCTAGTTCTTTCTTATCTTTC
```

```
C_11711720D_40 ABCB1c.3095 G>A
TATTTAGTTTGACTCACCTTCCCAG[C/T]ACCTTCTAGTTCTTTCTTATCTTTC
```

After running paired assays for triallelic SNPs in separate reactions on the same gDNA samples, examine the cluster plots in TaqMan® Genotyper™ Software. Sample calls may need to be adjusted, and the results of each assay compared to determine the true sample genotype, as detailed on page 64.

TaqMan® DME Assays for genotyping adjacent SNPs

For certain highly studied DME targets, two SNPs are located adjacent to one another, complicating the SNP genotype analysis given that for each assay, the probes will fail to bind target sequences when the adjacent SNP is present. However, when the adjacent SNPs are present in only 3 haplotypes, these SNPs can be interrogated similarly to triallelic SNPs using two assays (see "TaqMan® DME Assays for genotyping triallelic SNPs" on page 23).

Life Technologies currently provides assay sets to two highly studied SNPs that have an adjacent, less frequently detected SNP. In each case, the minor alleles of each SNP are not found together in the same haplotype. The assays and targets are shown in :

Table 4 Assays and targets

Gene	SNP ID	TaqMan DME assay	Allele name
CYP2C19	rs4244285	C__25986767_70	CYP2C19*2 681G>A
	rs6413438	C__30634128_10	CYP2C19*10 c.680C>T
CYP2C9	rs1057910	C__27104892_10	CYP2C9*3 c.1075A>C
	rs56165452	C__30634131_20	CYP2C9*4 c. 1076T>C

Example: CYP2C19*2,*10 adjacent SNP assays

Only 3 haplotypes have been observed for the CYP2C19 *2 and *10 SNPs (**http://www.cypalleles.ki.se/cyp2c19.htm**): the rare *10 c.680T allele and the *2 c.681A do not occur on the same chromosome. Thus, these adjacent SNPs can be analyzed similarly as for triallelic SNPs. When the *2 assay is run on a sample containing a *10 allele, the probes will fail to detect the *10-containing allele; the converse is true when the *10 assay is run on a sample containing a *2 allele. Below are shown the context sequences for each assay. After running paired assays for adjacent SNPs in separate reactions on the same gDNA samples, examine the cluster plots in TaqMan® Genotyper™ Software. Sample calls may need to be adjusted, and the results of each assay compared to determine the true sample genotype, as detailed on page 64.

CYP2C19*2 c.681G>A C__25986767_70

TTCCCACTATCATTGATTATTTCCc[A/G]GGAACCCATAACAAATTACTTAAAA

CYP2C19*10 c.680 C>T C__30634128_10

TTTCCCACTATCATTGATTATTTCC[C/T]gGGAACCCATAACAAATTACTTAAA

***10 *2**

TTTTCCCACTATCATTGATTATTTCC[C/T][G/A]GGAACCCATAACAAATTACTTAAA

Gender assays

Gender-specific assays can be included in PGx studies to aid sample tracking. The TaqMan® SNP Genotyping Assay, C_990000001_10, targets a gender-specific polymorphic region in the amelogenin gene that is commonly used in forensic sex determination tests. A 6 base deletion occurs in the X-specific amelogenin gene, which is detected by the VIC® dye probe, whereas the FAM™ dye probe detects Y-chromosome sequences. In genotyping experiments, male samples will run in the heterozygous cluster position and female samples will run in the VIC® homozygote

cluster. Note that some males lack the Y-specific amelogenin gene, and they will type as female. Thus, the C_990000001_10 assay should be run in combination with a Y-chromosome assay to identify any mistyped samples. For more information, go to **http://www.cstl.nist.gov/strbase/Amelogenin.htm**.

An example of a Genotyping Bar Code Y-chromosome assay that can be used: C___1083232_10 to the polymorphic rs2032598 SNP in USP9Y. Only male samples will amplify with this assay; female samples will cluster with the no template controls (NTCs).

Clinical research targets

TaqMan® SNP Genotyping Assays to clinical research targets are often included in PGx studies. These assays are found within the predesigned TaqMan® SNP Genotyping Assays collection and are searchable on the Life Technologies web site by NCBI dbSNP rsSNP ID. Note that common names for disease-associated alleles (e.g. Factor V Leiden) are not yet search terms. Public web sites that can be used to identify the rsSNP ID for common disease alleles include:

- Online Mendelian Inheritance in Man® (OMIM®): **http://omim.org/**
- SNPedia (a wiki investigating human genetics): **http://snpedia.com/**

In addition, TaqMan® SNP Genotyping Assays to commonly tested clinical research targets are included in the PGx Common Markers file that can be downloaded from **www.lifetechnologies.com/pgx**.

Unavailable DME and clinical research targets

TaqMan® SNP Genotyping Assays are an ideal technology for interrogation of most DME and clinical research target polymorphisms, offering highly specific target amplification and allele discrimination. However, there are some polymorphisms that are not well-suited for TaqMan® assay development. These include targets that:

- Reside in highly polymorphic genomic regions (polymorphisms interfere with amplification in some samples),
- Share high sequence identity with another genomic region (base differences are not available for specific assay development),
- Are microsatellite polymorphisms, or
- Are base deletions within a homopolymer sequence.

A list of commonly requested DME and clinical research targets that are not good candidates for TaqMan® Assay design and suggestions for alternative technologies to use are included in the PGx Common Markers file (**www.lifetechnologies.com/pgx**.)

Custom TaqMan® SNP Assays

Targets of interest that are not covered by the current Life Technologies TaqMan® SNP Genotyping collection can be submitted to Custom TaqMan® SNP Assay design.

The Custom TaqMan® Assay Design Tool (CADT) is available on the Life Technologies website (**www.lifetechnologies.com**). Order a custom TaqMan® SNP Genotyping Assay by first entering a sequence with the SNP in brackets, for example [A/G], then submitting the chosen target sites for assay design. Upon notification of successful assay design by email, click the link in the message and add the desired custom assays to your shopping basket.

CADT can be used to design assays targeting biallelic SNPs or insertion/deletion polymorphisms and multi-nucleotide polymorphisms (MNPs) that are 6 bases or fewer in length. This tool can also be used to input and order primer and probe sequences of assays that have already been designed that contain FAM™ or VIC® labels and MGB-NFQ quenchers.

Note that sequences must be SNP and repeat-masked before submission to CADT. Additionally, the genome-uniqueness for assays must first be established, because custom assays are not compared to the genome (e.g. by BLAT or BLASTn) to determine target specificity. Any target on the "unavailable list" (described on page 25) should not be submitted to CADT, because an assay may be designed but it will fail to function properly.

For targets that present assay design challenges, contact our fee-for-design custom assay design service at **custom.solutions@lifetech.com**.

Custom TaqMan® Copy Number Assays

Targets of interest that are not covered by the extensive Life Technologies human TaqMan® Copy Number Assay collection can be submitted to Custom TaqMan® Copy Number Assay design. The GeneAssist™ Copy Number Assay Workflow Builder is available on the Life Technologies website (**www.lifetechnologies.com**). This tool can be used to search for predesigned assays and to design custom plus assays (SNP masking and genome quality checks are included) or other custom assay types including submission of primer and probe sequences for previously designed FAM™ dye MGB-NFQ probe assays. All assay types, including requisite TaqMan® Copy Number Reference Assays, can also be ordered through the tool.

References

Gaedigk A, Bradford LD, Alander SW, and Leeder JS. 2006. CYP2D6*36 gene arrangements within the cyp2d6 locus: association of CYP2D6*36 with poor metabolizer status. *Drug Metab Dispos* 3:563-9.

He Y, Hoskins JM, McLeod HL. 2011. Copy number variants in pharmacogenetic genes. *Trends Mol Med.* 17:244-51.

Oscarson M., McLellan RA, Asp V, Ledesma M, Bernal Ruiz ML, Sinues B, Rautio A, and Ingelman-Sundberg M. 2002. Characterization of a novel CYP2A7/CYP2A6 hybrid allele (CYP2A6*12) that causes reduced CYP2A6 activity. *Hum Mutat.* 20:275-83.

Ramamoorthy A, Flockhart DA, Hosono N, Kubo M, Nakamura Y, and Skaar TC. 2010. Differential quantification of CYP2D6 gene copy number by four different quantitative real-time PCR assays. *Pharmacogenet Genomics*. 20:451-4.

Sim, S.C. and Ingelman-Sundberg, M. 2010. The Human Cytochrome P450 (CYP) Allele Nomenclature website: a peer-reviewed database of CYP variants and their associated effects. *Hum Genomics* 4: 278–281.

3 Ordering information

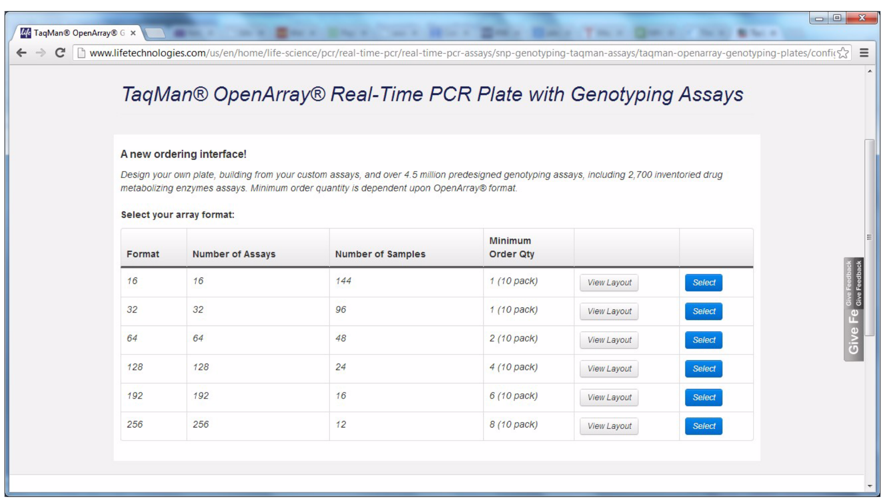
This chapter covers configuring and ordering OpenArray® plates using the online TaqMan® OpenArray® Configurator tool and ordering single tube assays. It also provides tables of reagents required for PGx genotyping experiments. The chapter covers:

Order custom OpenArray® plates

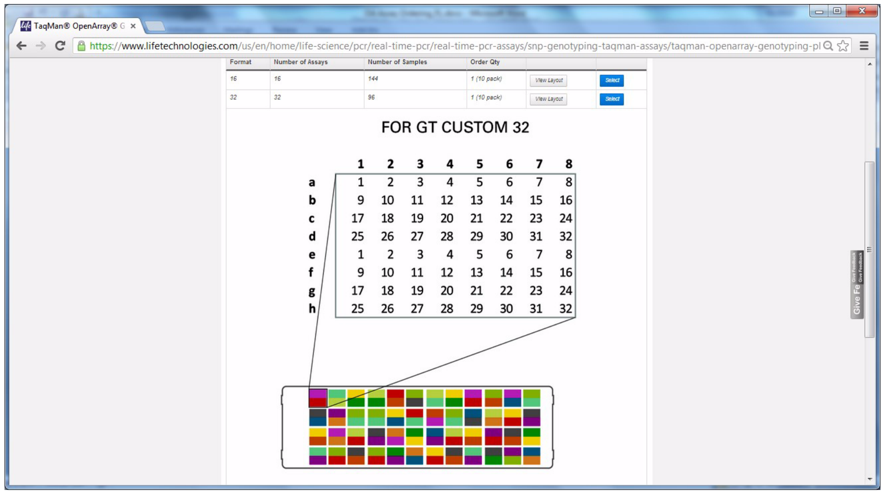
Design and order custom QuantStudio® 12K Flex TaqMan® OpenArray® Genotyping plates, which are delivered with assays dried down in the through-holes you specify, using the TaqMan® OpenArray® Plate Configurator.

To order custom panels of TaqMan® Genotyping Assays pre-plated on OpenArray® plates:

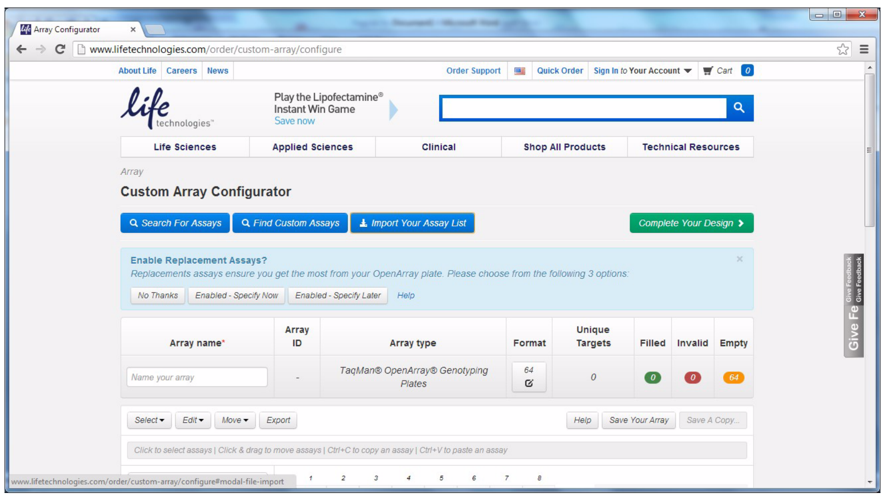
1. Navigate to the TaqMan® OpenArray® Real-Time PCR Plate with Genotyping Assays web page found at the Life Technologies website (**www.lifetechnologies.com**; Life Sciences ▸ TaqMan® Real-Time PCR Assays ▸ TaqMan® SNP Genotyping Assays ▸ OpenArray® Plates ▸ Configure a TaqMan® OpenArray® Genotyping Plate). The page contains a table of the available OpenArray® custom formats ranging from 16-256 assays per plate.



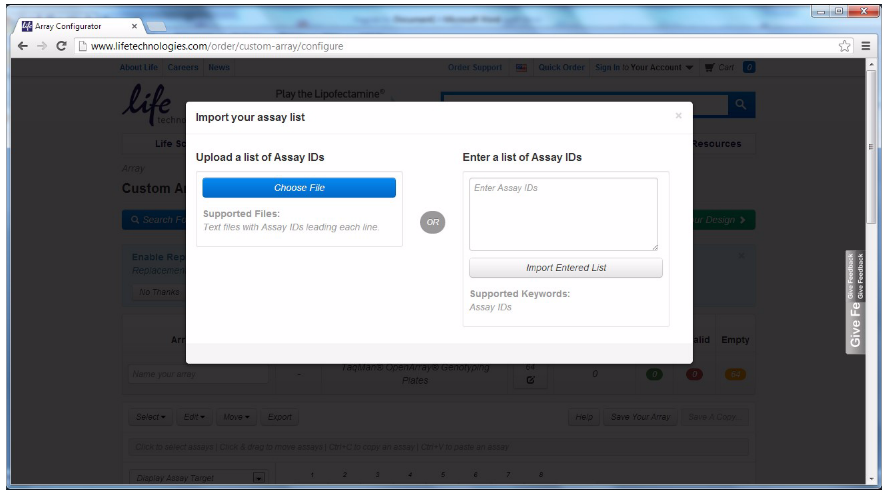
Click **View Layout** to visualize the assay format in the OpenArray® plate.



2. Click **Select** to enter the plate configurator. Search for assays, find custom assays, or import your predefined assay list.

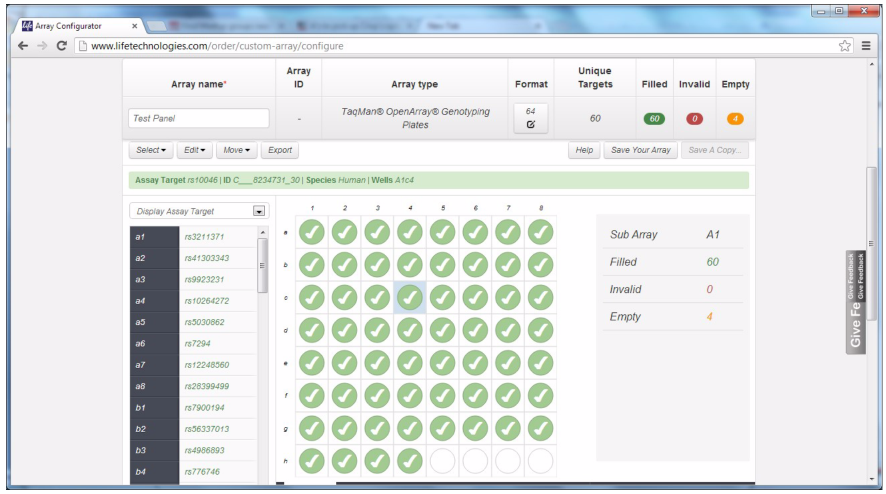


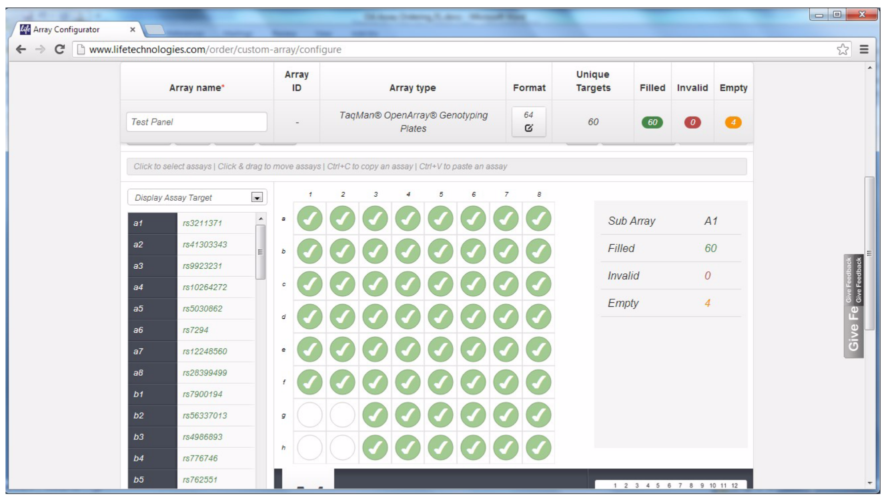
3. Upload your assay list. You can upload your assays from a tab-delimited text file (.txt) or paste the IDs in the Enter a list of Assays IDs search box and click **Import Entered List**.



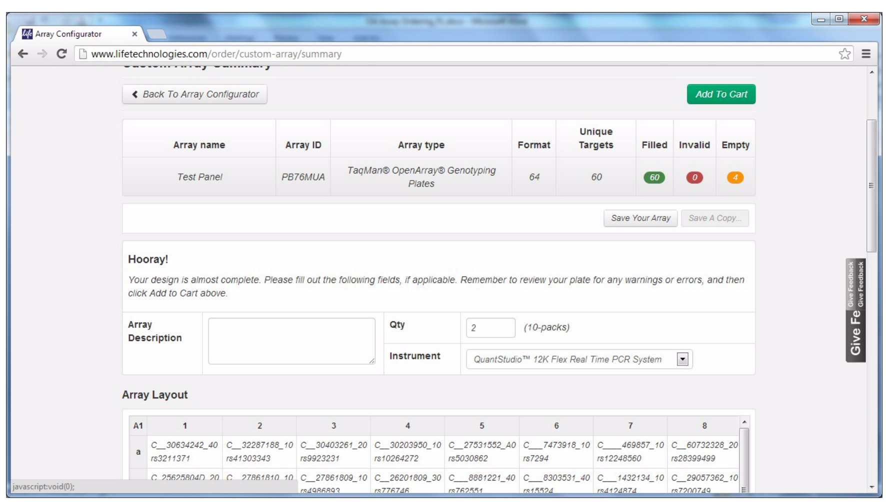
After you import the assays, the assays are automatically populated into the through-holes diagram for each subarray. You can rearrange the assay position by dragging and dropping the assay into the desired destination.

In the following example, we moved assays from the positions g1, g2, h1 and h2 into positions h5 through h8.





4. Click **Complete Your Design** to continue to the next step in the ordering process.



5. Select **QuantStudio 12K Flex Real Time PCR System** from the Instrument drop-down menu. Enter in the number of 10-packs for your order and your Array description. You can also click **Save Your Array** if you want to complete your order later or save it for future orders.

6. Click **Add to Cart** to add your OpenArray® plates to your shopping cart, where you can begin the checkout process to complete your order.

Table 5 Storage conditions for TaqMan® OpenArray® Genotyping Plates

If the TaqMan® OpenArray® Genotyping Plate is...	Storage Conditions
Frozen, unopened	Store at –20°C until the expiration date provided on the product label.
Thawed, unopened	Store at room temperature for up to 24 hours.
Thawed, opened	Store at room temperature for up to 1 hour.
Loaded and sealed, pre-thermal cycling	Store at room temperature, in the dark, for up to 24 hours.
Loaded and sealed, post-thermal cycling	Store at 4°C, in the dark, for up to 72 hours.

Order single-tube TaqMan® Assays

Chapter 2, "Background and tools for assay content selection" on page 13, provides instructions for searching for predesigned TaqMan® SNP and Copy Number assays. Chapter 2 also provides resources such as lists of commonly used and recommended specialty PGx assays. Methods of ordering assays are briefly described here.

Following a predesigned assay search, assays may be selected and added to a shopping cart for ordering. Order passing custom assays through the product-specific design tools. Select the scale of predesigned or custom assays before adding them to your cart or editing them in the cart (note that DME assays come in small scale only). Alternatively, assays may be ordered through the Quick Order web page, accessed from the upper right-hand corner of Life Technologies web pages, using the part number and Assay ID.

The TaqMan® SNP Genotyping Assay and TaqMan® Copy Number Assay products and part numbers are provided in the tables below.

Table 6 TaqMan® SNP Genotyping Assays

Product	Scale	Number of reactions		Assay mix formulation	Part no.	
		384-well	96-well		Human	Non-human
Predesigned SNP	Small	1500	300	40x	4351379	4351384
	Medium	5000	1000	40x	4351376	4351382
	Large	12000	2400	80x	4351374	4351380
DME	Small	750	150	20x	4362691	-
Custom SNP	Small	1500	300	40x	4331349	4332077
	Medium	5000	1000	40x	4332072	4332075
	Large	12000	2400	80x	4332073	4332076

- DME assays are available only as small scale inventoried product. Predesigned and custom SNP assays are made-to-order and are available in multiple scales.
- Assays with Human part numbers undergo functional testing on a panel of 20 unrelated Coriell cell line gDNA samples from 3 populations (African American, Caucasian, and Japanese) before shipment upon first order.
- Assays with Non-human part numbers include predesigned SNP assays that are designed to mouse SNPs and custom SNP assays designed to any non-human organism. Non-human part numbers can also applied to assays to human targets that would fail the human assay functional test (e.g. Y-chromosome SNP assays fail as 8 of 20 samples are female and will not amplify).

Table 7 TaqMan® Copy Number Assays (made-to-order)

Scale	Concentration	Number of reactions		Part no.†		
		384-well, 10 µL	96-well, 20 µL	Pre-designed assays	Custom Plus assays	Custom assays
Small	20X	720	360	4400291	4442487	4400294
Medium	20X	1500	750	4400292	4442520	4400295
Large	60X	5800	2900	4400293	4442488	4400296

† Only human assay part numbers are listed in this table.

Table 8 TaqMan® Copy Number Reference Assays (inventoried)

Product	Concentration	Number of reactions		Part no.
		384-well, 10 µL	96-well, 20 µL	
Human assays				
TaqMan® Copy Number Reference Assay RNase P, 750 Reactions	1 tube, 20X	1500	750	4403326
TaqMan® Copy Number Reference Assay RNase P, 3000 Reactions	4 tubes, 20X	6000	3000	4403328
TaqMan® Copy Number Reference Assay TERT, 750 Reactions	1 tube, 20X	1500	750	4403316
TaqMan® Copy Number Reference Assay TERT, 3000 Reactions	4 tubes, 20X	6000	3000	4403315

Reagents

TaqMan® Genotyping Master Mix is recommended for optimal performance with TaqMan® SNP Genotyping Assays and TaqMan® Copy Number Assays run on 96- or 384-well plates (this master mix cannot be used with OpenArray® plate).

Available volumes for TaqMan® Genotyping Master Mix (2X)	Part no.
1-Pack; one 10-mL bottle	4371355
2-Pack; two 10-mL bottles	4381656
Single Bulk Pack; one 50-mL bottle	4371357
Multi Bulk Pack; two 50-mL bottles	4381657

4 Prepare DNA samples

This chapter covers recommended procedures for DNA isolation, quantification, and optional preamplification:

Isolate and purify genomic DNA

Use the MagMAX™ DNA Multi-Sample Ultra Kit (Cat. nos. A25597 and A25598) for rapid, high-throughput isolation of high-quality genomic DNA from whole blood or buccal swab samples. The kit uses MagMAX™ magnetic bead technology, ensuring reproducible recovery of PCR-ready DNA suitable for OpenArray® analysis.

Refer to Appendix A on page 79 of this user guide for complete instructions for use of the kit (also available at the product web page at **www.lifetechnologies.com**):

- *MagMAX™ DNA Multi-Sample Ultra Kit User Guide – High-throughput isolation of PCR-ready DNA from buccal swabs (*Pub. no. MAN0010293)
- *MagMAX™ DNA Multi-Sample Ultra Kit User Guide – High-throughput isolation of PCR-ready DNA from whole blood (*Pub. no. MAN0010294)

Follow the recommendations and guidelines for specimen collection and storage in the appropriate user guide.

Quantify DNA samples

Quantify the isolated DNA by one of the following methods. Prior to quantification, mix the samples well to ensure sample homogeneity, especially if samples have been stored.

- **Standard curve analysis (recommended).** Use the TaqMan® RNase P Detection Reagents Kit (Cat. no. 4316831) for human gDNAs. Use your own human DNA samples or the TaqMan® DNA Template Reagents (PN 401970) to create a standard curve. Refer to Appendix B “Sample quantification using the RNase P Detection Reagents Kit” on page 88, or *Creating Standard Curves with Genomic DNA or Plasmid DNA Templates for Use in Quantitative PCR* (Pub. no. 4371090).
- **UV absorbance measurements.** Use a NanoDrop® or other comparable instrument. Pure gDNA should have an A_{260}/A_{280} ratio of approximately 1.8–2.0.
- **Fluorometric analysis.** Use a Qubit® dsDNA BR or HS Assay Kit.

Note: Standard curve analysis is the most accurate quantitation method whereas A_{260} and A_{280} readings can be used to assess both the concentration and the quality of the sample.

Conventionally, 50 ng/µL DNA stock solutions are recommended for OpenArray® genotyping experiments and A_{260}/A_{280} ratios should be approximately 1.8–2.0. If samples are of low concentration (<10 ng/µL) or contain PCR inhibitors, samples may fail to amplify, may fail to cluster properly, and in the worst-case scenario, may provide incorrect genotypes (e.g., apparent loss of heterozygosity if only one chromosomal copy is predominantly amplified and a heterozygous sample clusters within a homozygous cluster). If sample preparations perform poorly on the OpenArray® platform with a number of assays, we recommend preamplifying samples (see "(Optional) Preamplify DNA samples"), which can serve both to generate higher concentrations of target amplicons and to dilute PCR inhibitors.

Split DNA samples for copy number experiments

Reserve a portion of DNA from the MagMAX™ preparation for the TaqMan® Copy Number Assay workflow. Dilute the samples to a suitable working concentration; the recommendation is 5 ng/µL for each sample. The required input for each 10 µL PCR is 10 ng of DNA. Additionally, each sample must be run in quadruplicate for each copy number assay.

For example, if you are running 5 copy number assays, prepare at least:

(# of assays) × (Vol. of 5 ng/µL DNA per 10 µL reaction) × (# of replicates) × 120% (for dead volume)

= (5 Assays) × (2 µL) x (4 replicates) x 120%

= 48 µL of each sample at a concentration of 5 ng/µL

These diluted samples can be stored at 4°C for immediate use or at –25°C to –15°C for long-term storage.

(Optional) Preamplify DNA samples

Overview

This abbreviated protocol provides guidelines for any user to perform a targeted multiplex preamplification of 1 to 256 SNP loci located within human genomic DNA samples. The protocol is compatible for use with TaqMan® SNP Genotyping Assays. The user will use a multiplex pool of TaqMan® SNP Genotyping Assays to simultaneously preamplify up to 256 target polymorphisms in a single reaction using a reduced amount of input DNA sample. The product of this multiplex targeted preamplification may then be used as the sample template input for SNP genotyping reactions with any of the individual TaqMan® SNP Genotyping Assays included in the multiplex preamplification assay pool. A custom OpenArray® Preamp Pool specific for your TaqMan® SNP assay panel can be ordered along with your OpenArray® plate order (please contact your sales representative for details).

Reagents

Reagent	Catalog no.
TaqMan® PreAmp Master Mix	4391128
OpenArray® PreAmp Pool	4485255†
Genomic DNA samples	Supplied by user
1X TE Buffer, pH 8.0	12090015
Nuclease-free Water	Major laboratory supplier

† This product cannot be ordered directly from the Life Technologies website. For information on ordering an OpenArray® Preamp Pool, please contact your Life Technologies sales representative.

Procedure

Guidelines for DNA sample concentration

- Genomic DNA samples at a concentration up to 25 ng/µL may be preamplified without dilution, but the ideal range is 0.4 ng/µL to 4.0 ng/µL. DNA with concentrations outside this range may work, but DNA samples at a concentration >30 ng/µL may fail to amplify well because of the presence of PCR inhibitors. High concentration DNA samples may yield good results after dilution.
- The preamplification protocol was developed using genomic DNA samples within a starting concentration range of 0.4 ng/µL to 4.0 ng/µL (gives 0.5 ng to 5 ng total in the preamplification reaction).

  1 ng of human genomic DNA = 300 genomic copies, 150 copies of each allele in a heterozygote.
- Preamplification of DNA at concentrations <0.4 ng/µL is not recommended, due to the potential for stochastic events arising from low target number in the early rounds of the preamplification and qPCR reactions.
- Optimal performance will be achieved if the starting concentration of genomic DNA samples is near the middle of the working range (2.5 ng/µL). Refer to Appendix B "Sample quantification using the RNase P Detection Reagents Kit" on page 88 for a recommended protocol to accurately quantify your genomic DNA samples.

Perform the preamplification

1. Prepare the individual preamplification reactions in a 96-well reaction plate.

   For each individual genomic DNA sample, combine the reaction reagents as described in the table below. PreAmp Master Mix and OpenArray® PreAmp Pool can be prepared as a single cocktail and distributed to the plate in 3.75-µL aliquots. These volumes can be increased 2-fold. It is convenient to use plates with well volumes that can accommodate a 20X larger volume than the reaction for the 20-fold dilution step after preamplification.

Reagent	Stock conc.	Final conc.	Volume
TaqMan® PreAmp Master Mix	2X	1X	2.5 µL
OpenArray® PreAmp Pool	4X (0.20X each assay)	1X (0.05X each assay)	1.25 µL
Genomic DNA Sample	0.4 ng/µL to 4 ng/µL	0.1 ng/µL to 1 ng/µL (150–1500 copies)	1.25 µL
Total	—	—	**5.0 µL**

2. Firmly seal the reaction plate with a MicroAmp® Clear Adhesive Film.
3. Vortex the reaction plate for 10 seconds and spin briefly.
4. Run the preamplification cycling program on a GeneAmp® 9700 PCR System (silver or gold block) or Veriti® Thermal Cycler using the following temperature and time settings. The number of cycles can range from 10 to 14.

Stage	Step	Temp	Time
Hold	Activate	95°C	10 min
Cycling (10–14 cycles)	Denature	95°C	15 sec
	Anneal/Extend	60°C	4 min
Hold	Inactivate	99.9°C	10 min
Hold	—	4°C	up to 1 h or overnight

Dilute and store the preamplified product

Transfer the reaction plate from the thermal cycler to a container with ice. Keep the plate on ice until you are ready to dilute the preamplified product.

Dilute the preamplified product 1:20 using the following procedure:

1. Spin the reaction plate briefly prior to removing the film.
2. Remove film and add 95 µL of 1X TE Buffer to each well containing a preamplified product.
3. Firmly seal the reaction plate with a new MicroAmp® Clear Adhesive Film.
4. Vortex the reaction plate for 10 seconds and spin briefly.

Store the preamplified product at –25°C to –15°C.

Genotyping with preamplified product

Perform genotyping using the individual TaqMan® SNP Genotyping Assays following the standard protocol with the exception of substituting preamplified product for genomic DNA sample. It is not necessary to quantify or normalize preamplified product. The preamplified sample can be input directly into the reaction plate or further diluted in 1X TE Buffer to the desired concentration.

Note: Preamplification in OpenArray® experiments helps to ensure sufficient template copies in the 33-nL qPCR reaction. Typically there is not a need to preamplify samples if template in the qPCR reaction is present at >100 copies. At low template copy number, stochastic effects can dominate the reaction because the random events at each template molecule represent a large portion of the potential extension events. Typically, stochastic events are dominant at levels that are <10 template copies and are negligible in qPCR reactions with >100 template copies. Thus, the standard OpenArray® protocol for human SNP assays recommends starting with 50 ng/µL of genomic DNA. When diluted 50% in master mix and loaded into a 33-nL reaction chamber, the final template amount is 825 pg. This converts to 250 genomic copies (125 genomic copies for each allele in a heterozygote).

5 Prepare, run, and analyze OpenArray® PGx experiments

The information provided in this chapter includes streamlined procedures for running OpenArray® genotyping experiments on the QuantStudio® 12K Flex Real-Time PCR System, and for data analysis using the QuantStudio® 12K Flex system and TaqMan® Genotyper™ Software. The chapter covers:

About the OpenArray® chemistry protocol

This chapter provides summary procedures for performing experiments described in detail in the *QuantStudio® 12K Flex Real-Time PCR System OpenArray® Experiments User Guide* (Pub. no. 4470935).

The *QuantStudio® 12K Flex Real-Time PCR System OpenArray® Experiments User Guide* contains detailed instructions for performing experiments on the QuantStudio® 12K Flex Real-Time PCR System using TaqMan® OpenArray® plates. The guide contains a booklet on running the QuantStudio® 12K Flex OpenArray® Genotyping Starter Kit. In addition to describing how to run the starter kit, the booklet provides general information about genotyping experiments.

Track samples

OpenArray® plate loading overview

To load samples into a TaqMan® OpenArray® plate:

1. Pipet the samples into a 96-well reaction plate.
2. Transfer the samples from the 96-well reaction plate to an OpenArray® 384-Well Sample Plate using an adjustable or fixed pipette.
3. Transfer the samples from the OpenArray® 384-Well Sample Plate to the TaqMan® OpenArray® plate using the OpenArray® AccuFill™ System.

We recommend that you track the samples from the 96-well reaction plates to the 384-well sample plates using the OpenArray® Sample Tracker Software.

Note: This section provides brief procedures for using the OpenArray® Sample Tracker Software. For detailed procedures, refer to the *OpenArray® Sample Tracker Software Quick Reference Guide* (Pub. no. 4460657).

Enter experiment and sample information

In the Sample Tracker Software Properties window, enter general information about your experiment:

1. From the drop-down lists, make the following selections:

Drop-down list	Selection
Experiment type	**Genotyping**
OpenArray® Plate	The appropriate TaqMan® OpenArray® plate format
Pipettor	Appropriate pipettor: **Fixed** or **Adjustable**

2. If you have added a serial number or barcode to the OpenArray® 384-Well Sample Plate, enter the serial number or scan the barcode using a barcode reader.
3. Manually enter sample information from the 96-well reaction plates into the OpenArray® Sample Tracker Software or import a .csv file as described in the *OpenArray® Sample Tracker Software Quick Reference Guide* (Pub. no. 4460657).
4. Go to the sample mapping section on the software to begin the export process.

The OpenArray® Sample Tracker Software automatically maps the sample locations from the 96-well reaction plates to the appropriate locations in the 384-well sample plates and TaqMan® OpenArray® plates.

Export sample information

Next, export sample information in preparation for setting up plates with QuantStudio® OpenArray®AccuFill™ Software (see “Load samples using the OpenArray® AccuFill™ instrument” on page 42). Click the **Sample Mapping** button in the left bar in the Sample Tracker Software and export the sample information in table (*.csv) format:

1. Select plates to export as *csv files:
   - *(Recommended)* **384-well Plate.** Use this file with the QuantStudio® OpenArray® AccuFill™ Software to create a loaded SNP plate file (*.spf).
   - *(Optional)* **OpenArray® Plate n.** Use this *.csv file to import setup information into the QuantStudio® 12K Flex Software.

     Note: For OpenArray® Plate n, n refers to the number for the OpenArray® plate export. For example you can export OpenArray Plate 1, OpenArray Plate 2, OpenArray Plate 3, or OpenArray Plate, depending on what the customer wants to export.

   All plates are saved to individual *.csv files in the export directory. The OpenArray® Sample Tracker Software automatically assigns the file names.

2. Label and mark the plate. Using a fine-tip marker:
   - Label the 384-well sample plate with a unique identifier or use barcoded plates (Pub. no. 4453929).
   - Mark the 48-well sections of the 384-well sample plate that you will transfer the samples to from the 96-well reaction plates, using the tracking information that you obtained when you entered experiment and sample information and chose plates to export.

Transfer samples to OpenArray® 384-well sample plates

1. Thaw the 96-well reaction plate containing prepared gDNA samples at room temperature. Mix the gDNA samples by vortexing, then spin for 1 minute at 1000 rpm.
2. Review the concentration of the normalized gDNA samples. The recommended starting concentration for human gDNA samples is 50 ng/µL.

   Note: For optimal results, it is important to normalize all gDNA samples in an experiment. For human gDNA, make sure the samples are close to the recommended starting concentration of 50 ng/µL.
3. Mix the 2X TaqMan® OpenArray® Genotyping Master Mix by gently inverting the bottle 10 times.
4. Based on the layout you determined, load the 384-well sample plate:
   a. Add the master mix to the 384-well sample plate.
   b. Using a 12-channel pipette, transfer the normalized gDNA samples from the 96-well reaction plate to the 384-well sample plate.

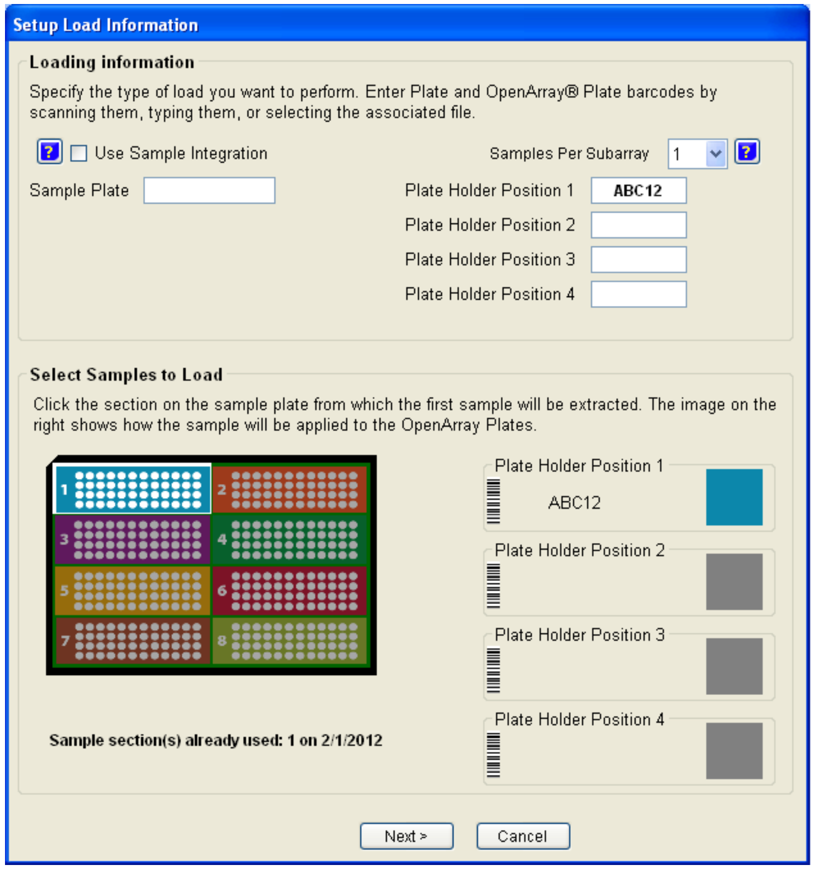
Component	Volume†, when transferring to:		
	Format 64‡	Format 16	Format 32
2X TaqMan® OpenArray® Genotyping Master Mix	2.5 µL	1.5 µL	2.0 µL
Normalized gDNA sample (human gDNA starting concentration = 50 ng/µL)	2.5 µL	1.5 µL	2.0 µL
Total volume	**5.0 µL**	**3.0 µL**	**4.0 µL**

† One well of a 384-well sample plate corresponds to one subarray of an TaqMan® OpenArray® plate. The number of subarrays required depends on the format of the TaqMan® OpenArray® plate.
‡ Starter kit experiment and larger formats

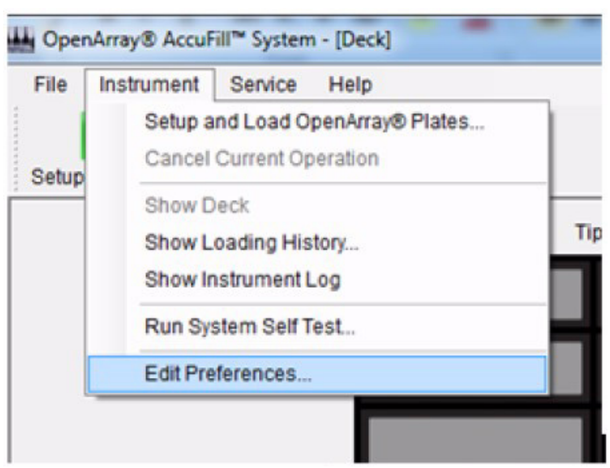
5. Cover the sample plate with foil. Vortex gently to mix, then centrifuge for 1 minute at 1000 rpm to eliminate bubbles. Mark the filled 48-well sections on the foil.
6. Place the sample plate on ice for up to 1 hour.

Load samples using the OpenArray® AccuFill™ instrument

1. On the OpenArray® AccuFill™ instrument software, click **Setup & Load**. In the Sample Plate field, browse to and open the *.csv file that contains the 384-well sample plate layout.



2. Set up sample integration in the QuantStudio® OpenArray® AccuFill™ Software:
   a. Launch QuantStudio® OpenArray® AccuFill™ Software and go to **Instrument ▸ Edit Preferences** as shown below:

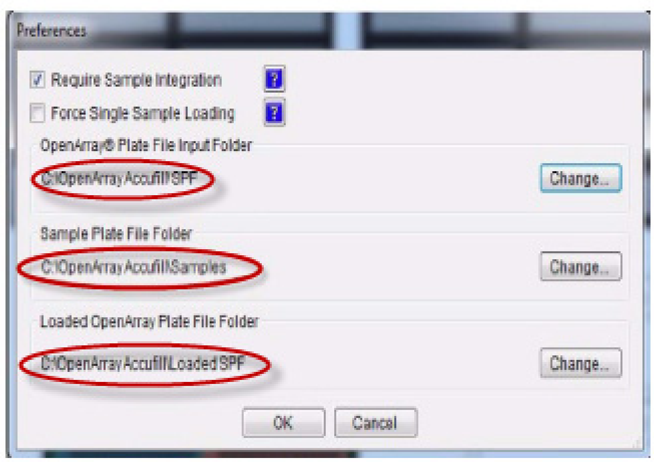


b. In the Preferences dialog box, check **Require Sample Integration**.

c. Create the directories shown in the Location column in the table below, then create shortcuts to these folders on the desktop.

Folder	Location	Contents
Input	*drive:*\Program Files\Applied Biosystems\OpenArray AccuFill\SPF	*.spf files that are downloaded from the web at **www.lifetechnologies.com/OA-platefiles**.
Sample Plate	*drive:*\Program Files\Applied Biosystems\OpenArray AccuFill\Samples	Contains sample *.csv files
Loaded OpenArray Plate	*drive:*\Program Files\Applied Biosystems\OpenArray AccuFill\Loaded SPF	Integrated *.spf files with sample names. The QuantStudio® OpenArray® AccuFill™ Software automatically places the integrated *.spf file with sample names in this folder (after the QuantStudio® OpenArray® AccuFill™ Software run). The resulting *.spf file includes the sample names.

d. Click Change to select the folders created for the Input, Sample Plate, and Loaded OpenArray® Plate folders:



3. Enter the data for the OpenArray® plates. For the first plate:

a. Select **1** from the Samples Per Subarray drop-down list.

b. In the plate holder Position 1 text field, enter the 5-character alphanumeric serial number of the OpenArray® plate you will load into the first position of the plate holder. You can:
   - Click **Browse**, then navigate to and open the plate setup file (*.spf) that corresponds to the OpenArray® plate. The software automatically displays the serial number in the Plate Holder Position 1 field.
   - Scan the serial number (barcode) located on the OpenArray® plate.
   - Type the serial number.

c. Thaw the OpenArray® plates for 15 minutes at room temperature. (Do not leave them at room temperature for more than 2 hours before use.)

d. Place a thawed sample plate into the plate holder.

   Hold the OpenArray® plate by the edges and place it into the plate holder with the barcode face up and to the left.

IMPORTANT! When you integrate a SampleID.csv into a plate setup file and enter the serial number by scanning or typing, the plate setup file must be located in the following directory:

```
drive:\Program Files\Applied Biosystems\OpenArray
AccuFill\Sample
```

Otherwise, the software will not be able to locate the file. The *drive* is the computer hard drive on which the QuantStudio® OpenArray® AccuFill™ Software is installed (unless a custom installation is performed, the default drive is C:).

Note: The AccuFill™ Software uses the serial number to access the appropriate plate setup files. During an instrument run, information in the plate setup files is used to populate the Assays screen in the QuantStudio® 12K Flex Software. As you enter the serial number, it is reflected in the representation of the OpenArray® plates in the lower section of the window.

4. Repeat step for the remaining OpenArray® plates, then click **Next**.
5. Place tip boxes, OpenArray® plates, and sample plate on the deck of the AccuFill™ instrument. Also, ensure the waste bin is empty.
6. Follow the window prompts until the instrument begins to load the array.
7. Once the loading is complete, place the loaded OpenArray® plate onto the plate press.
8. Peel protective layers from the lid and place onto the loaded OpenArray® plate on the plate press.
9. Engage the plate press and allow the plate to sit in the press for 20 seconds.
10. Remove the assembled OpenArray® plate from the plate press and add immersion fluid. Load immersion fluid until there is a small bubble that is enough to just fill one of the square windows near the alphanumeric plate ID.
11. Apply the plug to the port and tighten until the black handle breaks off from the screw.

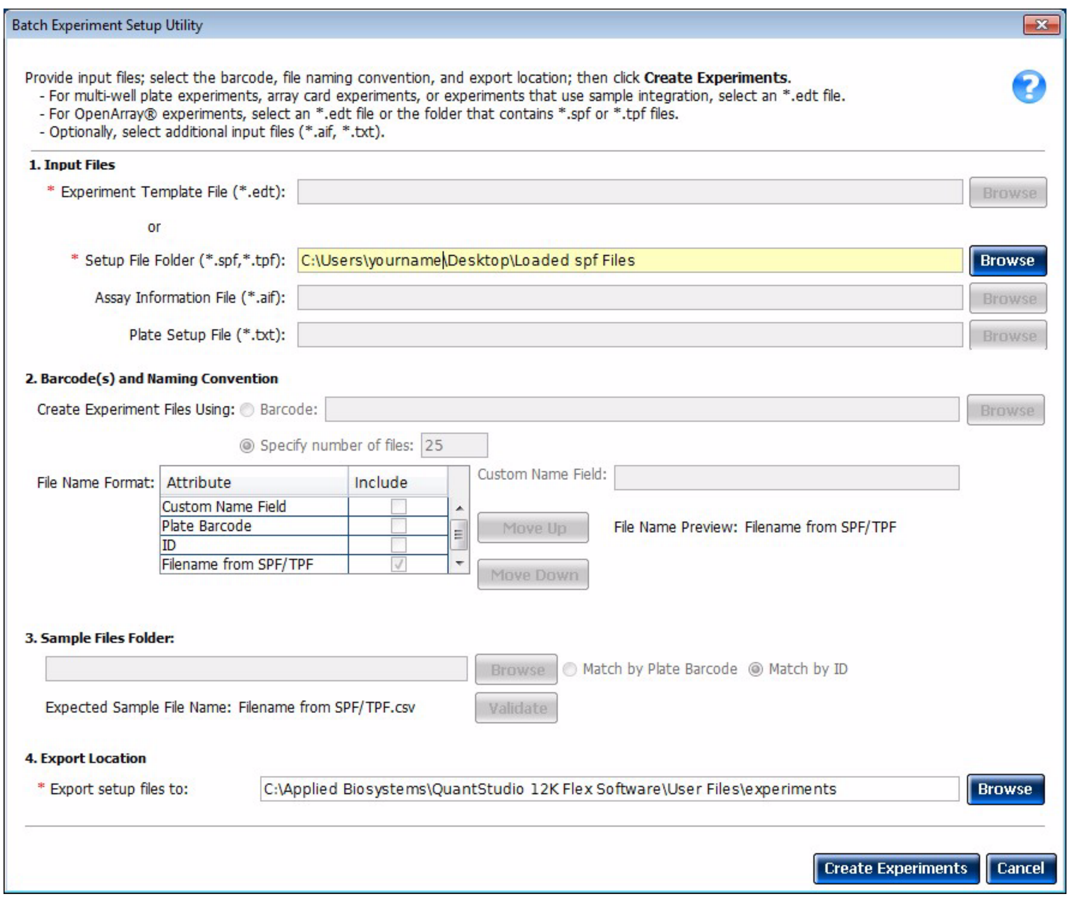
For more information please refer to *Applied Biosystems® QuantStudio® 12K Flex Real-Time PCR System: OpenArray® Plate Quick Reference Guide* (Pub. no. 4478673).

Create a batch of experiments in *.eds file format

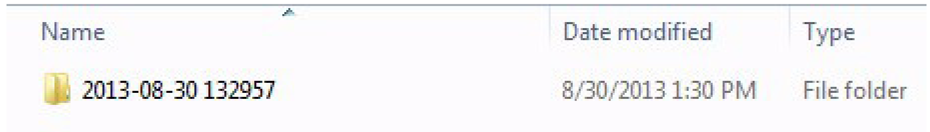
The following steps must be performed after sample integration using the AccuFill™ instrument to load the OpenArray® plate(s). Loaded spf files created during this process are required so that DNA sample locations are included. Although other options are available for batch file creation the steps recommended below describe the easiest workflow.

Note: Before using this workflow, the Setup Folder direction should be set under *drive*\Program Files\AppliedBiosystems\OpenArray AccuFill\Loaded SPF.

1. With the instrument powered on, start the QuantStudio® Software by double-clicking its icon.
2. On the uppermost tool bar click **Tools ▸ Batch Experiment Setup**.
3. In the next window under Input Files ▸ File Setup Folder, click **Browse** and select the following directory:
   `drive:\Program Files\AppliedBiosystems\OpenArrayAccuFill\Loaded SPF`
4. Under Export Location click **Browse** and select the following directory:
   `drive:\Applied Biosystems\QuantStudio 12K Flex Software\User Files\experiments`
5. Click **Create Experiments** at the bottom of the window.

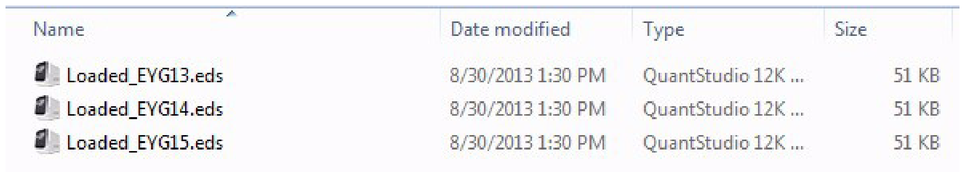


The experiments folder will now contain a directory named with a date and time stamp:



Name	Date modified	Type
2013-08-30 132957	8/30/2013 1:30 PM	File folder

The folder will contain one .eds file corresponding to each loaded spf file:



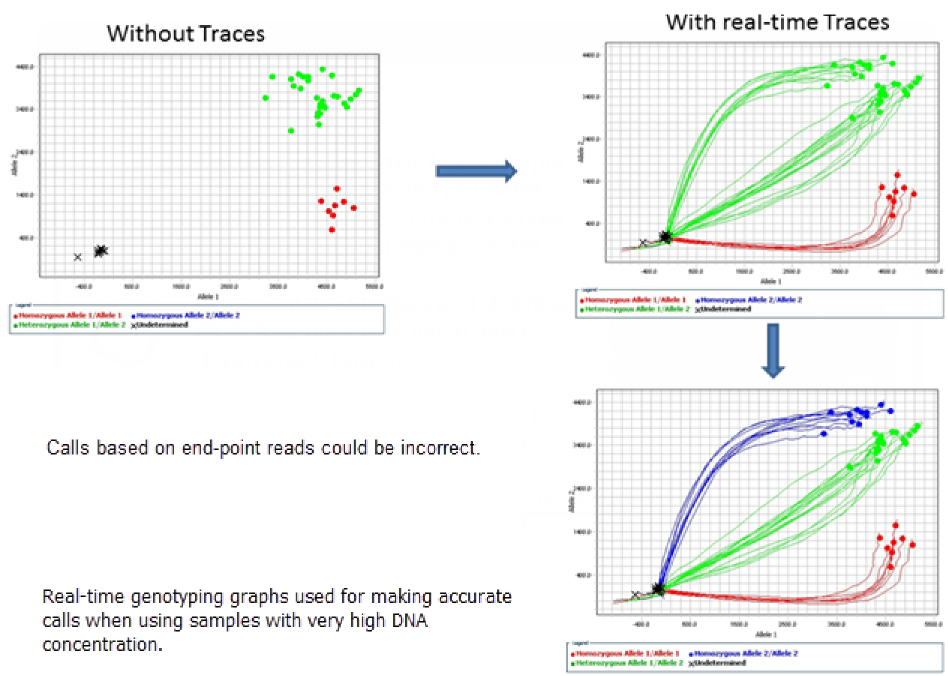
Name	Date modified	Type	Size
Loaded_EYG13.eds	8/30/2013 1:30 PM	QuantStudio 12K ...	51 KB
Loaded_EYG14.eds	8/30/2013 1:30 PM	QuantStudio 12K ...	51 KB
Loaded_EYG15.eds	8/30/2013 1:30 PM	QuantStudio 12K ...	51 KB

For more information about batch files click on the **?** in the top right corner of the Batch Experiment Setup Utility window to link to the relevant section of the *QuantStudio® 12K Flex Software Help*.

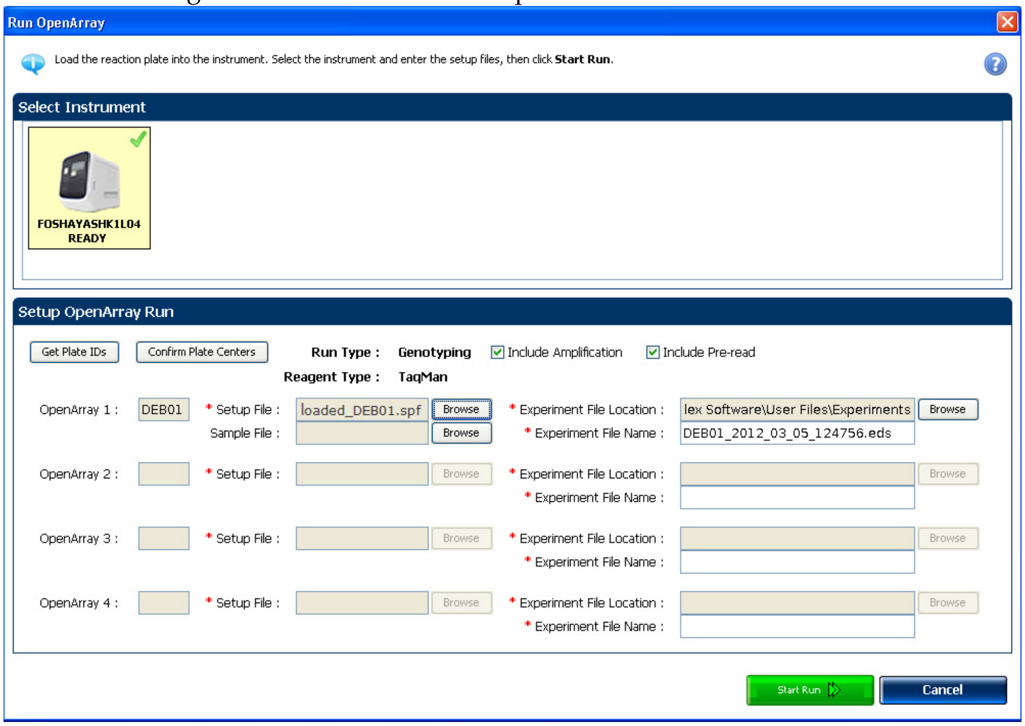
Run OpenArray® SNP genotyping experiments

Real-time vs. end-point genotyping experiments

It is strongly recommended that users run all OpenArray® plates on the QuantStudio® instrument in real-time experiment mode. If you run the OpenArray® plates on a thermal cycler outside of the instrument, you cannot observe genotype data over time to assess the accuracy genotype calls by observing the location of a given sample relative to others throughout all cycles. The example below shows where the DNA concentration is higher than the recommended amount.



The default run type for genotyping experiments is real-time mode, indicated by the check mark in the Include Amplification box shown below. The default settings also have a check mark in the Include Pre-read box; this setting is strongly recommended to correct for background fluorescence on the plate.



For additional information about genotyping experiments refer to *Booklet 2 - QuantStudio® 12K Flex OpenArray® Genotyping Starter Kit* in *Applied Biosystems™ QuantStudio® 12K Flex Real-Time PCR System OpenArray® Experiments User Guide* (Pub. no. 4470935). This document provides information about genotyping experiments in general in addition to describing how to run the starter kit.

Analysis settings in the QuantStudio® 12K Flex Software

Analysis setup

After the run has completed, click the analysis settings button. In the analysis settings window, there will be a data analysis settings portion near the top of the window, as shown below:

Each of the analysis settings is described in :

Table 9 Analysis settings

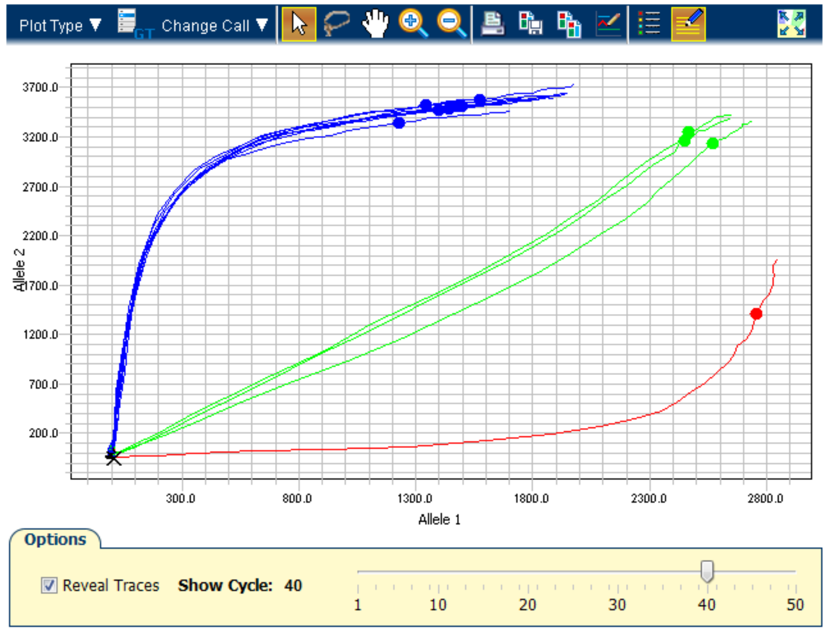
Analysis type	Explanation
Post PCR	Select if you do NOT want to use data from the pre-PCR read to determine genotyping calls; just the post-read data will be used.
Pre-PCR and Post-PCR	Select if you included the pre-PCR read in the run and you want to use the data to do a post-read minus pre-read calculation to determine genotyping calls. This calculation removes noise that may result from fluorescent materials in the system.
Real-time Rn	Select if you included amplification in the run and you want to use the reporter (Rn) data from the cycle 40 (or a user-defined cycle) from the real time plots to determine genotype calls.
Real-time Rn - Median (Rna to Rnb)	Select if you want to use the subtracted median of the reporter (Rn) data from cycling stages a and b to determine genotype calls, where "Rna to Rnb" refers to all the cycles from the Start Cycle Number a to the End Cycle Number (Default values are a=3 and b=15). The median subtraction provides improved data accuracy by performing a baseline correction.

The analysis we recommend for the PGx application is the "Analyze Real-Time Rn – Median (Rna to Rnb)" setting. This setting will normalize for any run and system noise to improve data accuracy.

Click **Apply Analysis Settings** at the lower left corner of the analysis settings window after choosing the analysis settings. If you choose the recommended analysis setting, then you can turn on the real time trace option by clicking the icon highlighted in yellow below:

To view real-time traces, open the experiment. In the toolbar on the left select **Analysis ▸ Allelic Discrimination Plot**. Then select the box highlighted in yellow above. Click the plot to view the genotype calls by color.

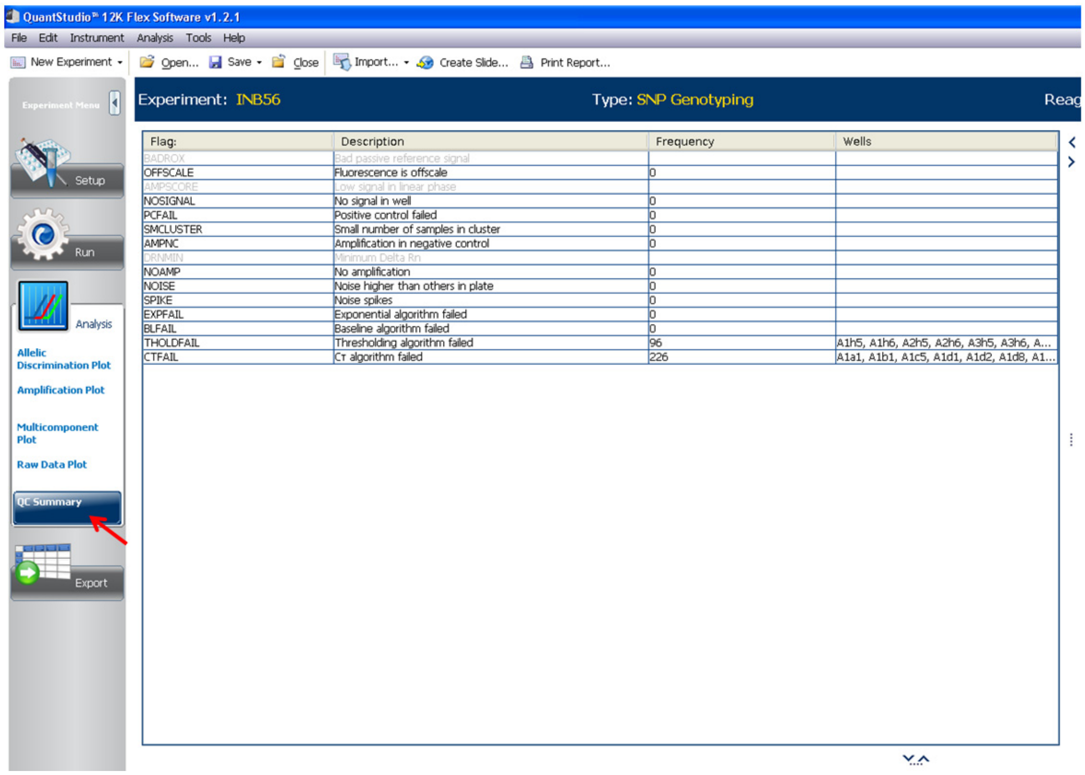
It can be difficult for the secondary analysis software (TaqMan® Genotyper™ Software) to make distinct genotype calls for assays with merging clusters (see example below). In these cases, the end-point cycle number can be manually adjusted to an earlier cycle using the Reveal Traces scroll bar, so that the clusters will have a greater degree of separation.



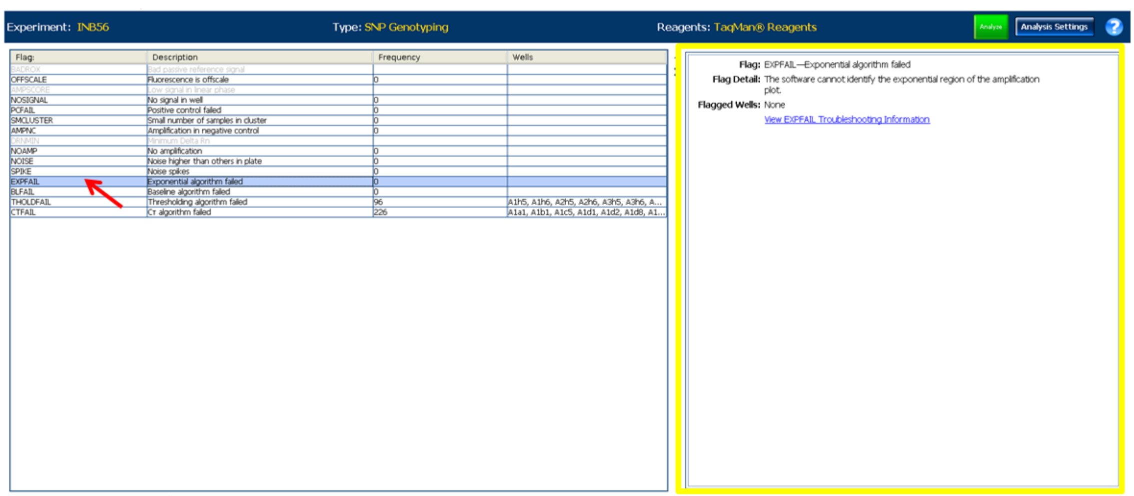
Save the run after all adjustments are made. Take note of the cycle number for each assay that has been adjusted. These numbers can later be entered into the TaqMan® Genotyper™ Software.

Note: A lower call cycle presently does not transfer to the TaqMan® Genotyper™ Software. TaqMan® Genotyper™ Software will re-set the cycle number to 50 cycles. Set up a separate study with the lower cycle number.

If you click the **QC Summary** tab in the analysis section on the left hand side of the software (as shown below), then you can view the QC flags for the run.



If you click each flag, it will bring up and explanation of what the flag means and a troubleshooting link on the right, as shown below:



Note: Do not be concerned about the CTFAIL flag for genotyping data. Some samples do not have both alleles present. This flag will be shown if the amplification curve from the absent allele does not meet quality metrics, but the amplification will be poor if an allele is absent.

For analysis, using the help of the real-time traces from the QuantStudio® 12K Flex v1.2 Software, go through the data by assay and manually call data if necessary, e.g., if any real-time traces do not show a smooth progression in signal throughout the run.

Transfer files to a user computer

Transfer files from QuantStudio® 12K Flex Software without a network connection

If the QuantStudio® 12K Flex is connected to a computer and the run has been downloaded to the computer, then the user can browse for the folder containing the run and download the file to storage media. If there is no computer and the instrument is not connected to the network, then a user can insert a USB drive into one of the USB ports in the front panel. The user can then use the touchscreen and press **Collect Results.** Search for the desired runs to be downloaded and touch the screen to select the run names. After the names have been highlighted, the user can press **Save to USB**.

Transfer files from QuantStudio® 12K Flex Software with a network connection

1. In the Home screen of the QuantStudio™ 12K Flex Software, click **Instrument Console**.
2. In the Instrument Console, select the desired QuantStudio™ 12K Flex Instrument from the list of instruments on the network, then click **Add to My Instruments**.
3. After the QuantStudio™ 12K Flex Instrument is added to your list, select it, then click **Manage Instrument**.
4. In the Instrument Manager, click **Manage Files**, then click **File Manager**:
5. In the File Manager screen, transfer the file(s) from the QuantStudio™ 12K Flex Instrument:
   a. In the Folders field, select the folder that contains the files that you want to download.
   b. In the Experiments field, select the files to download. To select multiple files, **Ctrl-click** or **Shift-click** files in the list.
   c. When you have selected the files that you want to download, click **Download**.
   d. In the Send experiment to instrument dialog box, select the folder to which you want to download the selected file(s), then click **Open**.

For more details on file transfers please refer to the networking chapter in the *Applied Biosystems QuantStudio® 12K Flex Real-Time PCR System Maintenance and Administration User Guide* (Pub. no. 4478673).

Perform analysis in TaqMan® Genotyper™ Software

For a detailed description of genotype analysis with TaqMan® Genotyper™ Software, please refer the *TaqMan® Genotyper™ Software Getting Started Guide* (Pub. no. 4448637).

Create a study in TaqMan® Genotyper™ Software

Create a new study or create a study from a template

To create a study, in the toolbar on the TaqMan® Genotyper™ Software Home screen, click **Create Study**.

Alternatively, create a study from a template file. Click **Create Study from Template**, then select and open a template file using the Select Template dialog box.

Note: See the *TaqMan® Genotyper™ Software Getting Started Guide* (Pub. no. 4448637) for details on creating study templates.

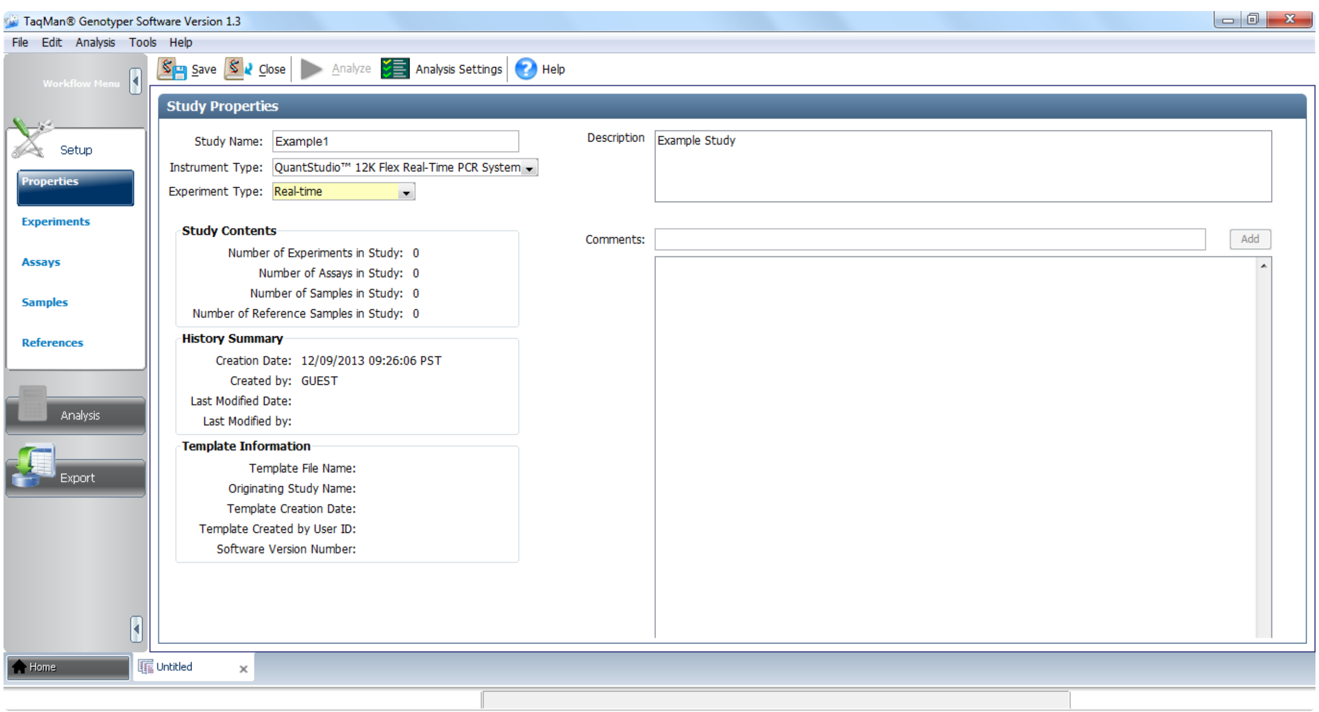
Enter study properties

1. In the Workflow Menu pane, select **Setup ▸ Properties** to open the Properties screen. (The Properties screen is the default view.)
2. Enter a study name.
3. Select **QuantStudio® 12K Flex Real-Time PCR System** from the Instrument Type drop-down menu.
4. Select **Real-time** from the Experiment Type drop-down menu:
5. (Optional) Enter a description of the study.
6. (Optional) In the Comments field, enter comments for the study, then click **Add**. The software records the comments, your user name, and the date and time you added the comments.

   Note: The Comments field allows you to enter detailed information about the study (for example, observations about the data, reasons why you made specific decisions, and so on). After you click Add, the comment is permanently recorded in the study (that is, the comment cannot be modified or removed) and the comment is included in any audit trails that are exported for the study.

   Note: You can enter comments at any time. You may prefer to enter comments after viewing and analyzing the data.

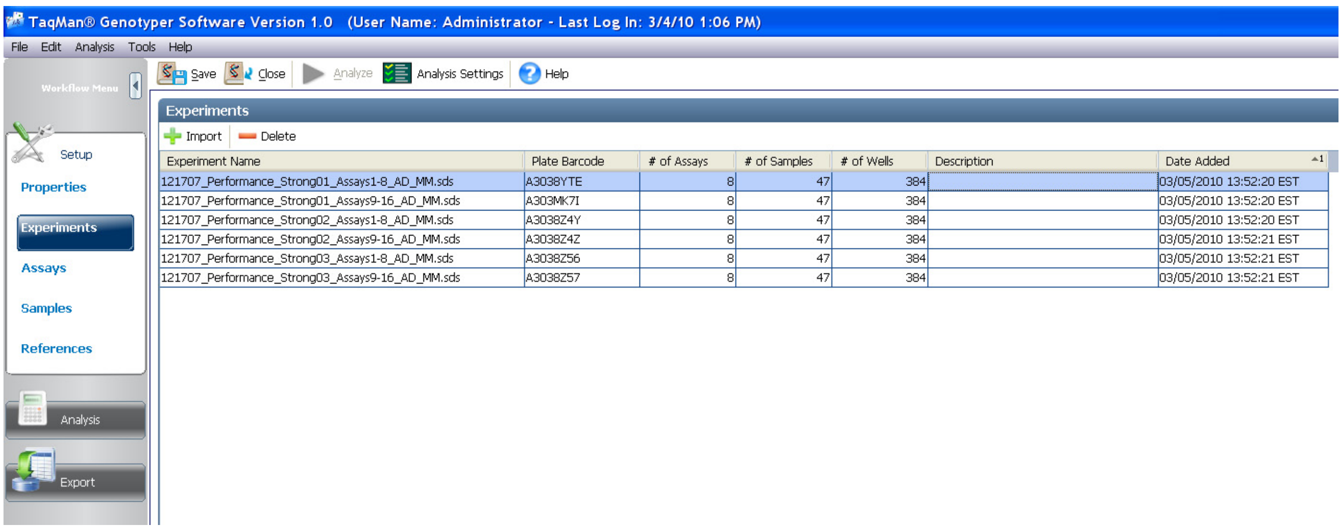
The Properties screen after study creation is shown below:



Import experiments into the study

1. In the Workflow Menu pane, select **Setup ▸ Experiments** to open the Experiments screen.
2. Click **Import**.
3. In the Import dialog box, browse to and select the experiment of interest. To import more than one experiment at a time, press **Ctrl** or **Shift** when you select the experiments. Be sure that all experiments meet the criteria defined in the *TaqMan® Genotyper™ Software Getting Started Guide* (Pub. no. 4448637).
4. Click **Import**.

The Experiments screen after import is shown below:



Import assay information files

The assay information file that you can download from the web at **www.lifetechnologies.com/OA-plate** contains important information, including the assay IDs, context sequence containing the SNP alleles and their association with reporter dyes, NCBI SNP reference IDs, and gene names. This assay information can be imported into TaqMan® Genotyper™ software. The SNP alleles will then be reported by the software, otherwise, the alleles will be reported as VIC® and FAM™ alleles.

1. In the Workflow Menu pane, select **Setup ▸ Assays** to open the Assays screen.
2. Click **Import**, browse to and select the assay information file of interest, then click **Import**.

   IMPORTANT! The assay information file must include an assay ID (in the Assay ID column) for each assay listed in the file. The software matches the assay IDs in the file with the existing assay IDs in the study.

3. If the assay information file contains information for an assay ID that is already in the study, the software prompts you to ignore the new information, replace the existing information, or cancel the import. Click the appropriate option.

4. View the imported assay information in the Assays screen. If the assay information file contains incorrect information, you can:
   - Edit the information in the Assays screen: Select the assay of interest, then click Edit to open the Edit Assay dialog box.
   - Edit the information in the assay information file, then re-import it.
   - Import a different assay information file to replace the incorrect information.

Add control identifiers

About control identifiers

Control identifiers identify the samples that the TaqMan® Genotyper™ Software uses as controls when analyzing a study. You can identify samples as:

- **NTC** (No-template control) – The well does not contain any template (DNA sample). The software automatically identifies any well in the study that has a sample ID of **NTC** as a no-template control.

  IMPORTANT! Life Technologies strongly recommends that you run two NTC wells with every assay in a study.
- **Negative Controls –** The well does not contain known template; that is, the well should display no amplification signal. (For example, the well may contain a non-target template, include an inhibitor, and so on.)
- **Positive Controls –** The well contains known template to generate a specific genotype call for an assay. You can specify positive controls for VIC®/VIC® (homozygous sample for the allele detected by VIC®), VIC®/FAM™ (heterozygous), or FAM™/FAM™ (homozygous for the allele detected by FAM™).

  Positive controls are not called by the software and cannot be manually called. In the Results screen, each positive control is identified as the genotype that was assigned to it in the Control Identifiers tab. This allows the software to preferentially use the positive control data points to help determine the calls of Unknown data points. We recommend that you carefully review the coordinates of positive control data points and omit these data points if they are not located in an expected position in the scatter plot.

  Positive controls are similar to reference samples in that the software can use both to bias the calls of Unknown data points. However, a positive control represents a well that physically contains known template and is included in one of the experiments in the current study. A reference sample can represent any well from any experiment in any study. For more information on reference samples, see the *TaqMan® Genotyper™ Software Getting Started Guide* (Pub. no. 4448637).

To add control identifiers:

1. In the toolbar, click **Analysis Settings** to open the Analysis Settings dialog box.
2. Select the **Control Identifiers** tab.
3. Select or deselect the O**verride Control Settings from Experiments** check box:

When this check box is:

- **Selected** – The software uses the control identifiers that you set in the Control Identifiers tab (steps and below), and overrides any control identifiers (tasks) that may have been set in the original experiment file. If a sample ID does not match a control identifier, the software assumes the sample is an Unknown.
- **Deselected** – The software uses the control identifiers (tasks) that were set in the original experiment file.

4. In the Study-Level Settings pane, enter the sample IDs to use as controls for all assays in the study. Use a comma to separate multiple control identifiers.

   Note: The Study-Level Settings apply to all assays in the study.

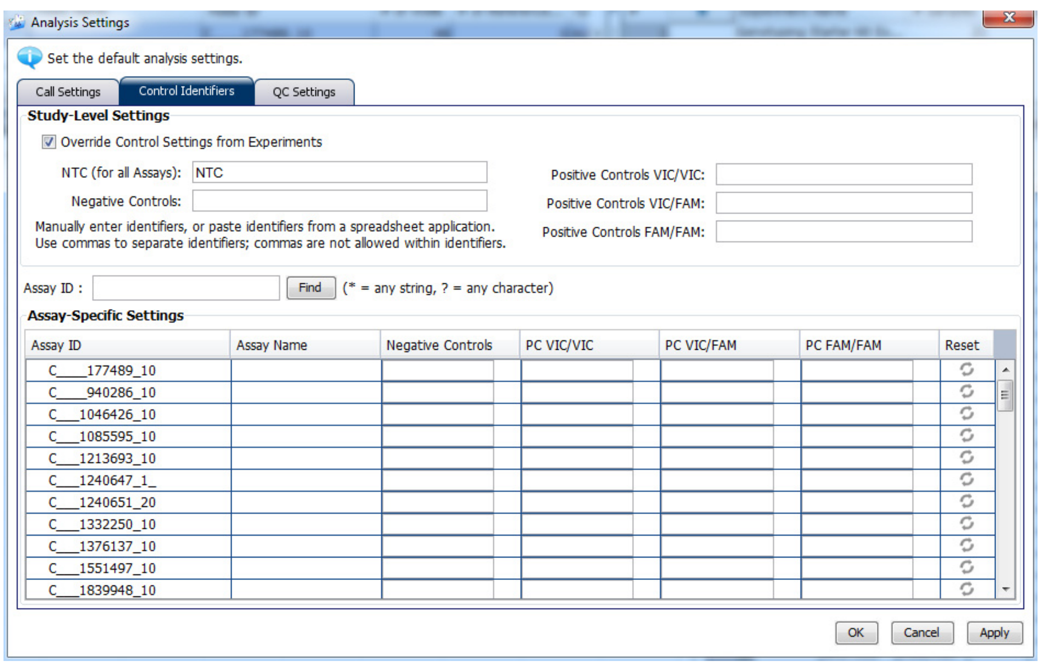
5. In the Assay-Specific Settings pane, enter the sample IDs to use as controls for individual assays in the study. Use a comma to separate multiple control identifiers.

   Note: By default, the Assay-Specific Settings are the same as the Study-Level Settings. If you modify the Assay-Specific Settings, your changes apply only to the selected assay (and override the Study-Level Settings for the selected assay). If needed, click the Reset symbol to reset the assay's settings to the Study-Level Settings.

6. Click **Apply** to save the changes.

The Analysis Settings dialog box with Control Identifiers window is shown below.

Note: When experiments are added to a study, assay IDs are automatically populated in this window. Assay names can be added by importing an assay information file to which an Assay Names column has been added.



Impact of analysis settings

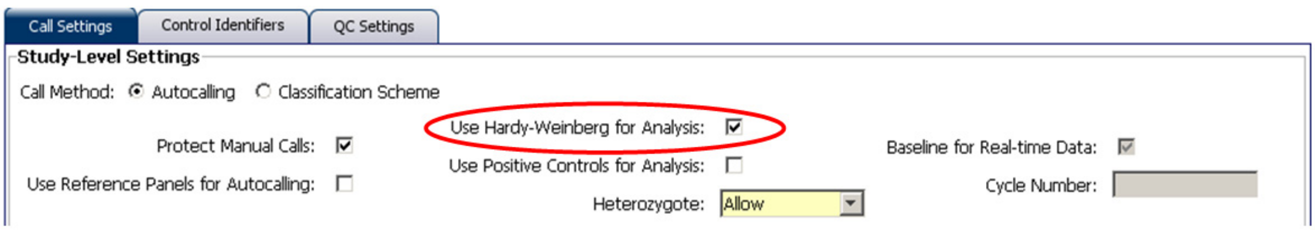
This section describes the impact of the following analysis settings in the Analysis Settings dialog box:

- Hardy-Weinberg for Analysis check box
- Heterozygote analysis option for sex chromosome targets

Note: In the toolbar, click **Analysis Settings** to open the Analysis Settings dialog box.

Hardy-Weinberg for Analysis check box

For targets expected to follow Hardy-Weinberg equilibrium, select Use Hardy-Weinberg for Analysis to favor genotype distributions based on the Hardy-Weinberg principle over other distributions and reduce the risk of incorrect calls.



Note: X and Y chromosome-specific SNP targets do not follow a Hardy-Weinberg distribution. SNP targets located in the pseudo-autosomal (PAR) region of the X and Y chromosomes behave like autosomal SNP targets and follow a Hardy-Weinberg distribution.

Note: The probes of the gender assay (C_990000001_10) target X- and Y- chromosome specific sequences in the amelogenin gene: males run as heterozygotes and females run as VIC® homozygotes. The VIC® probe detects a sequence in the gene found only on the X chromosome and the FAM™ probe detects a sequence in the gene found on the Y chromosome. Hardy-Weinberg rules will not apply and this box should be unchecked for this assay.

Heterozygote analysis options for sex chromosome targets

SNP targets on the:

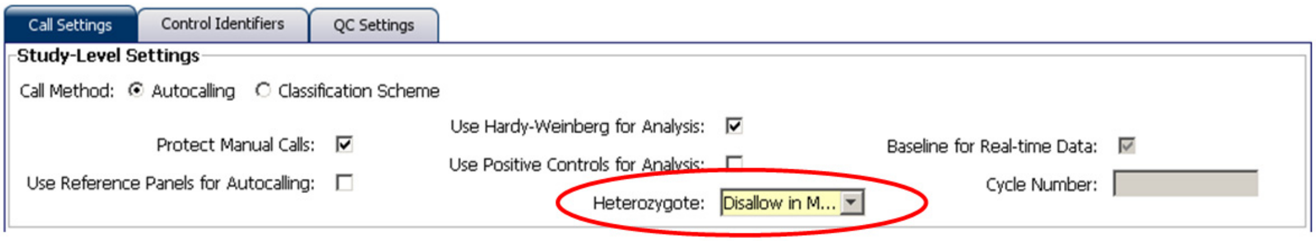
- X chromosome cannot be heterozygous in males because males carry only one copy of the X chromosome.
- Y chromosome cannot be heterozygous in males because there is only one copy of the Y chromosome.

Y chromosome-specific assays will not amplify female samples because females do not carry the Y chromosome.

For SNP targets on the X and Y chromosomes, select **Disallow in Males** from the Heterozygote drop-down menu to improve the accuracy of the genotype calls.

Note: SNP targets located in the PAR region of the X and Y chromosomes behave like autosomal SNP targets and males can be heterozygotes. The gender assay (C_990000001_10) target X- and Y- chromosome specific sequences in the amelogenin gene: males run as heterozygotes and females run as VIC® homozygotes. The Disallow in Males flag should not be set with these assay types.

The Disallow in Males drop-down menu is shown below:



Note: If you select Disallow in Males for other types of targets, the genotype call accuracy may be negatively impacted.

Set the QC settings

About QC settings

The QC settings are a collection of criteria that the software uses to analyze data in a study. You select the flags (criteria) that you want the software to use, and if applicable, enter a condition and threshold value appropriate for your laboratory.

There are two categories of criteria that the software uses to analyze data. The first category looks at concordance of data points, either to an expected call or to a replicate call. The second category looks at the data to meet a specified condition and threshold value. In either case, if the resulting data violates a criterion, a flag is generated.

By default, all flags are enabled in the software.

Set QC settings

1. In the toolbar, click **Analysis Settings** to open the Analysis Settings dialog box.
2. Select the **QC Settings** tab.
3. In the Well-Level QC Flags pane:
   a. Select the flags that the software uses to analyze the study at the well level.
   b. For each flag, select condition and threshold values that are appropriate for your laboratory:

QC flag	Description
Failed Control	A Failed Control flag can be raised for any data point that is identified as a control: NTC, Negative Control, or Positive Control. If the user-assigned control identifier (or task) for a data point is inconsistent with the call that would be assigned by the software algorithm to an Unknown with the same FAM™ and VIC® dye intensities, a flag is raised.
Genotype Quality	A Genotype Quality Low flag can be raised for any data point that is identified or tasked as an Unknown. If the quality value assigned by the software algorithm for a data point is below the threshold, a flag will be raised.
Low ROX™ Intensity	A Low ROX™ Intensity flag can be raised for any data point. If the ROX™ dye intensity determined by the software for a data point is below the threshold, a flag will be raised.
NTC FAM™ Intensity High	An NTC FAM™ Intensity High flag can be raised for any data point that is identified or tasked as an NTC. If the FAM™ dye signal intensity for a data point tasked as NTC is greater than the threshold, a flag will be raised.

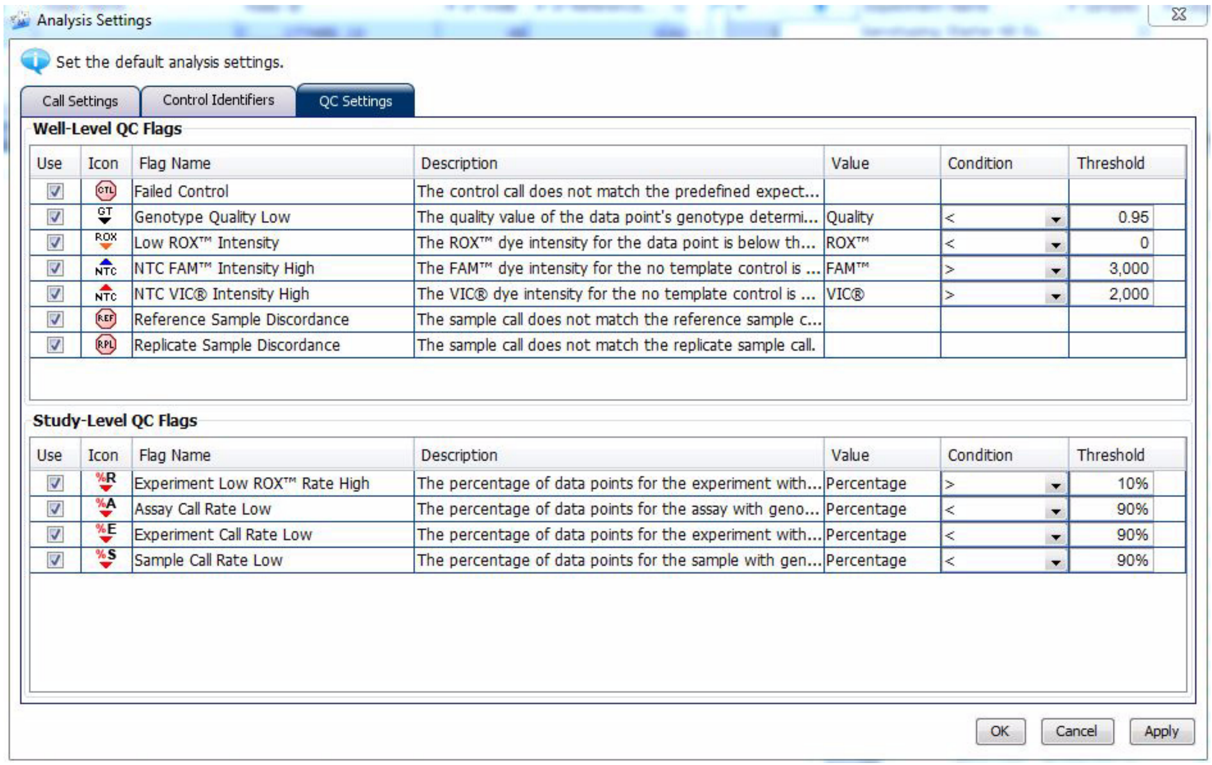
QC flag	Description
NTC VIC® Intensity High	An NTC VIC® Intensity High flag can be raised for any data point that is identified or tasked as an NTC. If the VIC® dye signal intensity for a data point tasked as NTC is greater than the threshold, a flag will be raised.
Reference Sample Discordance	A Reference Sample Discordance flag can be raised for any data point that is identified or tasked as an Unknown. If the software algorithm-assigned genotype for a data point is discordant with the genotype of a reference sample data point that has exactly the same sample/assay identification, a flag will be raised.
Replicate Sample Discordance	A Replicate Sample Discordance flag can be raised for any data point that is identified or tasked as an Unknown. If the software algorithm-assigned genotype for a data point is discordant with the genotype of a replicate sample data point that has exactly the same sample/assay identification, a flag will be raised. A flag will be raised for all data points that have the sample/assay identification, because the software cannot know which data point has the correct genotype.

4. In the Study-Level QC Flags pane:
   a. Select the flags that the software uses to analyze all assays in the study.
   b. For each flag, select condition and threshold values that are appropriate for your laboratory:

QC flag	Description
Experiment Low ROX™ Rate High	An Experiment Low ROX™ Rate High flag can be raised for any experiment. If the percentage of data points in an experiment with a low ROX™ dye intensity flag is greater than the threshold, a flag will be raised.
Assay Call Rate Low	An Assay Call Rate Low flag can be raised for any assay. If the percentage of Unknown data points with a genotype call for an assay is less than the threshold, a flag will be raised.
Experiment Call Rate Low	An Experiment Call Rate Low flag can be raised for any experiment. If the percentage of Unknown data points in an experiment with a genotype call is less than the threshold, a flag will be raised.
Sample Call Rate Low	A Sample Call Rate Low flag can be raised for any sample identified or tasked as an Unknown. If the percentage of assays with a genotype call for an Unknown sample is less than the threshold, a flag will be raised.

5. Click **OK** to save the changes and close the Analysis Settings dialog box.

The Analysis Settings dialog box with QC settings is shown below:



Well-Level QC Flags

Use	Icon	Flag Name	Description	Value	Condition	Threshold
☑	CTL	Failed Control	The control call does not match the predefined expect...			
☑	GT	Genotype Quality Low	The quality value of the data point's genotype determi...	Quality	<	0.95
☑	ROX	Low ROX™ Intensity	The ROX™ dye intensity for the data point is below th...	ROX™	<	0
☑	NTC	NTC FAM™ Intensity High	The FAM™ dye intensity for the no template control is ...	FAM™	>	3,000
☑	NTC	NTC VIC® Intensity High	The VIC® dye intensity for the no template control is ...	VIC®	>	2,000
☑	REF	Reference Sample Discordance	The sample call does not match the reference sample c...			
☑	RPL	Replicate Sample Discordance	The sample call does not match the replicate sample call.			

Study-Level QC Flags

Use	Icon	Flag Name	Description	Value	Condition	Threshold
☑	%R	Experiment Low ROX™ Rate High	The percentage of data points for the experiment with...	Percentage	>	10%
☑	%A	Assay Call Rate Low	The percentage of data points for the assay with geno...	Percentage	<	90%
☑	%E	Experiment Call Rate Low	The percentage of data points for the experiment with...	Percentage	<	90%
☑	%S	Sample Call Rate Low	The percentage of data points for the sample with gen...	Percentage	<	90%

Export analysis data

For detailed information about the exported file contents, refer to the *TaqMan® Genotyper™ Software Getting Started Guide* (Pub. no. 4448637).

1. In the Workflow Menu pane, select **Export** ▸ **Analysis Data**, then select **Basic** or **Advanced Export**.
2. Select the type of data to export (you can select both options at the same time):
   - **Analysis Results**
   - **Analysis Settings**
3. If you selected Analysis Results in step 2, select **Basic** or **Advanced** (for use with AlleleTyper™ Software, select **Advanced**).
4. Select the default, **Separate Files**, to export the data in separate files. All data types you selected in step 2 (Analysis Results and/or Analysis Settings) are exported separately in different files.
5. Click **Export preview**, then **Start Export** in the new window. The resulting export file is the data input file for AlleleTyper™ Software.

(Optional) Generate and import a reference panel

This section provides step-by-step procedures to:

- Generate a reference panel file (this page)
- Import the reference panel file (page 63)
- (If needed) Delete a reference panel file (page 63)

About reference panel files and reference samples

A reference panel file is a user-generated TaqMan® Genotyper™ Software file that contains reference samples.

Reference samples are data points in an experiment that you select to be representative of the clusters for an individual assay. You can identify, collect, and store reference samples for multiple assays in a reference panel file for use in current or future studies. After you import a reference panel file into a study, the software uses the reference samples as a guide for the calls of Unknown data points. The reference samples cannot be modified and are not calculated in the call rates.

Note: Reference samples are similar to positive controls in that the software can use both to guide the calls of Unknown data points. However, a positive control represents a well that physically contains known template and is included in one of the experiments in the current study. A reference sample can represent any well from any experiment in any study.

Generate a reference panel file

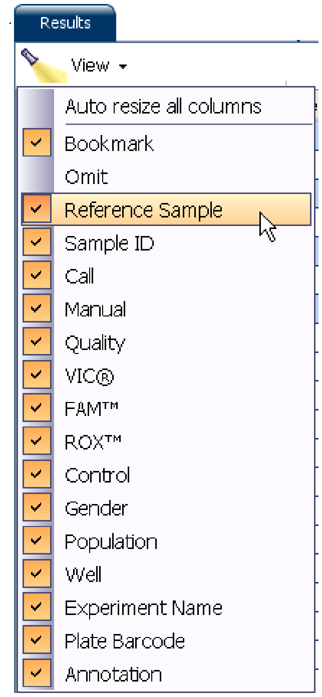
Before you can import a reference panel file into a study, you must first generate the file in the TaqMan® Genotyper™ Software:

1. In the TaqMan® Genotyper™ Software, open a study that contains data points that you want to use as reference samples.

   Note: You must select the data points at the assay level. The software doesn't allow you to select data points at the study level (that is, if you select a data point for one assay, it is not selected for all assays in the study).

2. In the Workflow Menu pane, select **Analysis ▸ Results** to open the Results screen.
3. Select the **Results** tab.

4. From the View drop-down menu, select **Reference Sample** to display the Reference Sample column in the Results table.

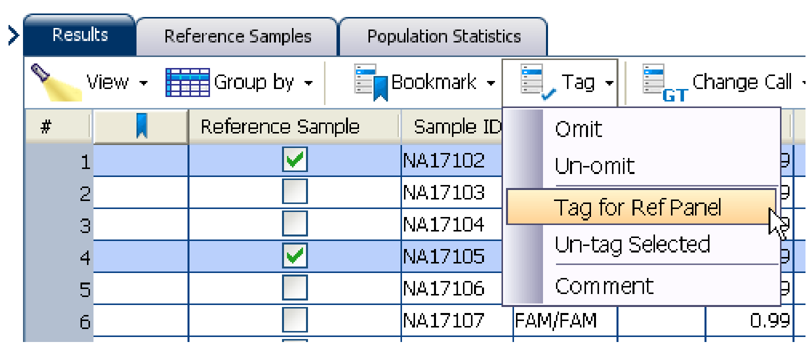


5. In the Results table or the scatter plot, select the samples that you want to use as reference samples:

   In the Results table:

   a. Select a sample in the Results table. To select more than one sample at a time, press **Ctrl** or **Shift**.

   b. From the Tag drop-down menu, select **Tag for Ref Panel**.

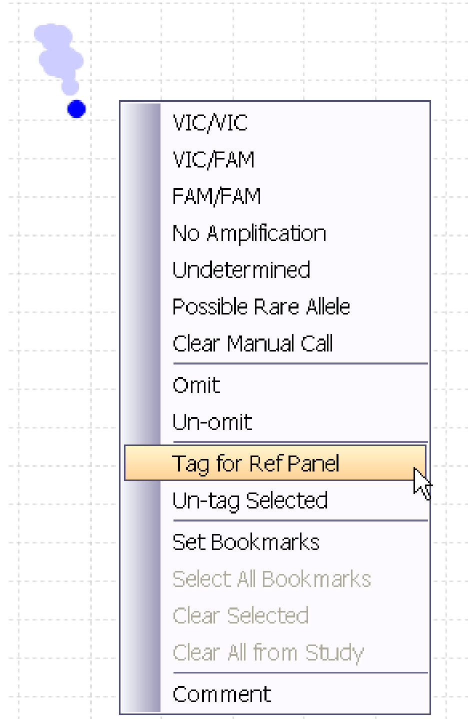


   Note: To remove a reference sample tag from a sample, you can either deselect the check box in the Reference Sample column or select the sample, then select **Un-tag Selected** from the Tag drop-down menu.

   In the scatter plot:

   a. Select a sample by drawing a box around the data point in the scatter plot.

b. Right-click in the scatter plot, then select **Tag for Ref Panel** from the menu.



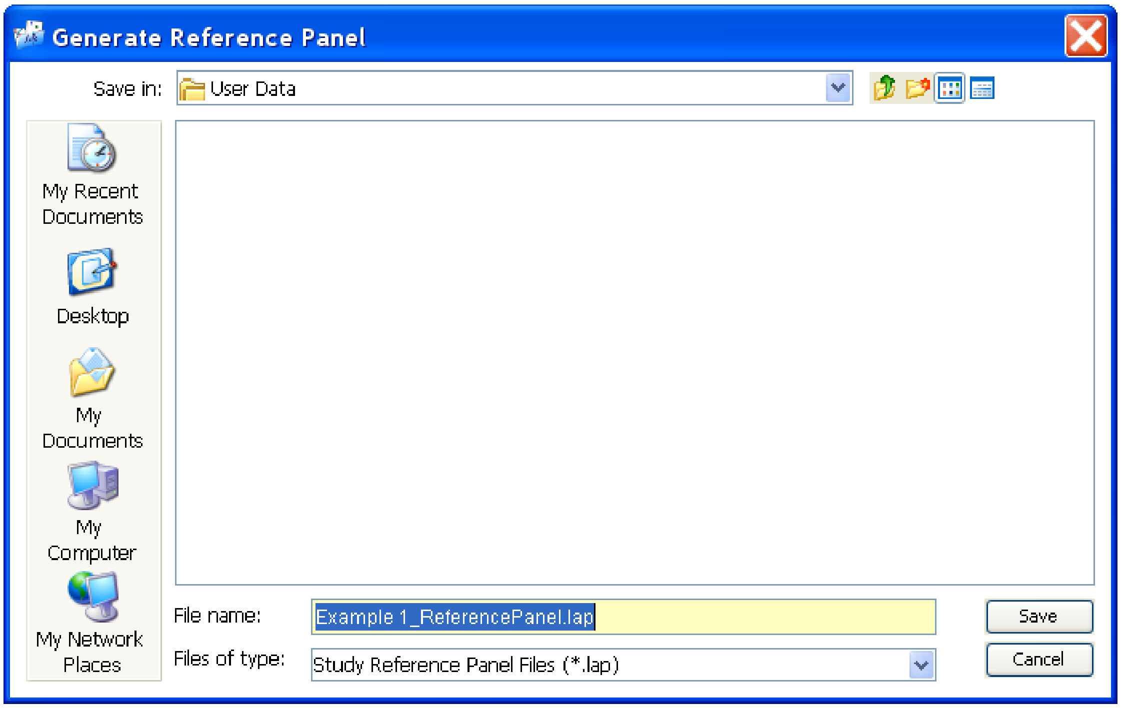
Note: To remove a reference sample tag from a sample, draw a box around the data point, right-click in the scatter plot, then select **Un-tag Selected** from the menu.

In the Reference Sample column in the Results table, checkmarks appear next to the selected samples.

6. Save the study.

7. From the menu bar, select **File ▸ Generate Reference Panel**.

8. In the Generate Reference Panel dialog box, browse to and select a save location, enter a name for the reference panel file (or accept the default name), then click **Save**.



Import the reference panel file

1. In the TaqMan® Genotyper™ Software, open a study that you want to import the reference panel file into.
2. In the Workflow Menu pane, select **Setup** ▸ **References** to open the References screen.
3. Click **Import.**
4. In the Import dialog box, browse to and select the reference panel file of interest, then click **Import**.

   Note: The software automatically analyzes the study whenever you import the reference panel file.
5. To view the imported reference samples for each assay:
   a. From the Workflow Menu pane, select **Analysis** ▸ **Results**.
   b. In the Results table, select the **Reference Samples** tab to see the listing of reference samples applied to an assay. For more information, see the *TaqMan® Genotyper™ Software Getting Started Guide* (Pub. no. 4448637).

(Optional) Delete a reference panel file

1. In the References screen, select the reference panel file to delete. To delete more than one file at a time, press **Ctrl** or **Shift** when you select the files.
2. Click **Delete,** then click **Yes** to confirm.

   Note: The software automatically analyzes the study whenever you delete a reference panel file.

Assays that require manual genotype calls

Special circumstance SNP and DME genotyping assays

While reviewing all project assay data in TaqMan® Genotyper™ Software is always advised, certain assays require manual examination of the data and adjustment of the genotype calls. Special circumstance SNP and DME genotyping assays include those that target polymorphisms that are in genes associated with CNV or target triallelic or adjacent SNPs.

TaqMan® DME genotyping assays to genes in copy number variation regions

As detailed in Chapter 2 on page 13, some DME Assays target polymorphisms in genes that exhibit Copy Number Variation including CYP2D6, CYP2A6, CYP2E1, GSTM1, GSTT1, and SULT1A1. Copy number variation analysis must be done in addition to genotyping with DME assays. The frequency of the CYP2D6, CYP2A6, CYP2E1, and SULT1A1 gene deletions are low, and samples that carry no copies of these genes will be rare. However, the frequency of deletion of GSTM1 and GSTT1 genes is very high in a number of populations and complete absence of these genes in individuals is common. For a given DME assay that targets a gene that can be deleted or duplicated, the following genotyping results are possible:

- If both copies of a gene are deleted in a sample (copy number of 0), samples will not be amplified and will run with NTCs. If a sample running near the NTCs has been called as undetermined, that call can be manually adjusted to “noamp”.
- If a sample contains 1, 2 or more than 2 copies of the gene and only one SNP allele is present, the samples will cluster together in a homozygous allele. Occasionally, clusters will show some splitting with the samples containing less target running closer to the NTCs and those with more target having more signal, but this is rare.
- If a sample carries more than 2 copies of a gene and both SNP alleles are present, it will fall within the heterozygous cluster or occasionally to one side or the other of it (i.e. the heterozygous cluster may exhibit some spreading). If a sample close to the heterozygous cluster has been called as undetermined, manually adjust the call to heterozygous.

TaqMan® DME genotyping assays to triallelic SNPs and adjacent SNP targets

As detailed in Chapter 2, triallelic SNP and adjacent SNP targets can be interrogated using a pair of TaqMan® assays. For triallelic SNPs, each assay contains one probe for the major SNP allele, which is labeled with the same reporter dye in both assays (e.g. VIC® dye), and one probe for one of the minor alleles, which is labeled with the second reporter dye (e.g. FAM™ dye). After running paired assays for triallelic SNPs in separate reactions on the same genomic DNA samples, the results of the 2 assays are compared to determine the sample genotype. For a given assay:

- Samples that are heterozygous for the alleles detected by an assay will run as heterozygous.
- Samples running in or near a homozygous cluster can be either a true homozygote for the reported allele, or can be a heterozygous for a reported allele and for the unreported SNP allele of a given assay. Samples having just one reported allele may run together with, or close to, those carrying two reported homozygous alleles. If a sample close to a homozygous cluster is called as undetermined, manually change the call to homozygous.
- Samples that are homozygous for the unreported allele may cluster with NTCs or may exhibit weak amplification due to probe nonspecific activity. If a weakly amplifying sample is called as undetermined, manually adjust the call to 'noamp'.

The TaqMan® Genotyper™ Software results can be exported and files imported into AlleleTyper™ for translation using a biallelic translation specific for the triallelic SNP assay pair. If AlleleTyper™ is not used for translation analysis, use the method exemplified in the table below to manually determine the sample genotypes.

The example translation table is for the ABCB1 c.3095G>T/A triallelic SNP rs2032582 assays. If AlleleTyper™ is not used for translation analysis, use the method exemplified in the table below to manually determine the sample genotypes. Note that the alleles reported by the ABCB1 SNP assays are given in the plus strand genome orientation whereas the ABCB1 gene maps to the minus genome strand. Thus the reported SNP assay alleles and the SNP cDNA annotations are the reverse complement of one another.

Table 10 Translation table for the ABCB1 c.3095G>T/A triallelic SNP rs2032582 assays

Genotype	C/A assay c.3095G>T, A893T	C/T assay c.3095G>A, A893S
ABCB1 c.3095G>T/A	C_11711720C_30	C_11711720D_40
G/G	C/C	C/C
G/A	C/C	C/T
A/A	No amp	T/T
G/T	C/A	C/C
T/A	A/A	T/T
T/T	A/A	No amp

Similarly pairs of assays are available for some adjacent SNP targets for which only 3 haplotypes are noted. For a given assay to one SNP allele:

- Samples that are heterozygous for the haplotypes detected by an assay will run as heterozygous.
- Samples running in or near a homozygous cluster can be either a true homozygote for the reported haplotype, or can be heterozygous for a reported haplotype and an unreported haplotype of a given assay. Samples having just one reported haplotype may run together with, or close to, those carrying two reported homozygous haplotypes. If a sample close to a homozygous cluster is called as undetermined, manually change the call to homozygous.
- Samples that are homozygous for the unreported haplotype may cluster with NTCs or may exhibit weak amplification due to probe nonspecific activity. If a weakly amplifying sample is called as undetermined, manually adjust the call to “noamp”.

Table 11 Translation table for the CYP2C19*2,*10 adjacent SNP assays. Note that because the *2 and *10 alleles have not been observed to occur on the same chromosome, diplotypes including the A-T haplotypes are not included.

Haplotype/ Haplotype	Diplotypes	*2 assay 681 G/A - 680C	*10 assay 681G - 680 C/T
	CYP2C19	C__25986767_70	C__30634128_10
G-C/G-C	*1/*1	G/G	C/C
G-C/G-T	*1/*10	G/G	C/T
G-C/A-C	*1/*2	G/A	C/C
G-T/G-T	*10/*10	noamp	T/T
G–T/A–C	*2/*10	A/A	T/T
A–C/A–C	*2/*2	A/A	noamp

(Optional) Single tube reintegration

If a sample fails to cluster well with other samples or fails to amplify properly with one or more assays (i.e. there are UND and NOAMP calls in the run), there is an option to rerun such samples using single tube DME assays on 96-well or 384-well plates. It is recommended to run control gDNA samples along with the unknown samples or use a reference panel (see “(Optional) Generate and import a reference panel” on page 60). The controls will help to provide more accurate genotype calling of the unknown samples.

6 Prepare, run, and analyze copy number experiments

This chapter covers:

Introduction to copy number analysis

Copy number variation must be assessed for DME genes that are known to exhibit copy number variation. For full details on running TaqMan® Copy Number Assay experiments, refer to the *TaqMan® Copy Number Assays Protocol* (Pub. no. 4397425). The protocol provides step-by-step instructions for performing and analyzing copy number variation quantitation experiments using TaqMan® Copy Number Assays and TaqMan® Copy Number Reference Assays for the ViiA™ 7 Real-Time PCR System. The same instructions apply for running experiments on the QuantStudio® 12K Flex Real-Time PCR System.

How the assays work

TaqMan® Copy Number Assays are run simultaneously with a TaqMan® Copy Number Reference Assay in a duplex real-time PCR reaction. The Copy Number Assay detects the target gene or genomic sequence of interest, and the Reference Assay detects a sequence that is known to exist in two copies in a diploid genome (for example, the human RNase P H1 RNA gene).

The number of copies of the target sequence in each test sample is determined by relative quantitation (RQ) using the comparative C_T ($\Delta\Delta C_T$) method. This method measures the C_T difference (ΔC_T) between target and reference sequences, then compares the ΔC_T values of test samples to a calibrator sample(s) known to have two copies of the target sequence. The copy number of the target is calculated to be two times the relative quantity, because the human genome is diploid.

In a copy number quantitation reaction, purified genomic DNA is combined with:

- The TaqMan® Copy Number Assay, containing two primers and a FAM™ dye-labeled MGB probe to detect the genomic DNA target sequence.
- The TaqMan® Copy Number Reference Assay, containing two primers and a VIC® dye-labeled TAMRA™ probe to detect the genomic DNA reference sequence.
- The TaqMan® Genotyping Master Mix, containing AmpliTaq Gold® DNA Polymerase, UP (Ultra Pure) and dNTPs required for the PCR reactions.

Reactions are run on an Applied Biosystems® Real-Time PCR System. After amplification, data files containing the sample replicate C_T values for each reporter dye can be exported from the real-time PCR instrument software and imported into a software analysis tool. Applied Biosystems® CopyCaller® Software is recommended for post-PCR data analysis of copy number quantitation experiments.

Prepare the reaction plates

Copy Number analysis is a sensitive application. For optimal results, use high quality purified and quantified gDNA samples. Life Technologies recommends using MagMax-purified samples for both OpenArray® and Copy Number Variation experiments. (See Chapter 4, “Prepare samples” on page 35.)

High quality, quantified sample DNA stocks of 5 ng/µL are recommended for copy number experiments (see “Split samples for copy number experiments” on page 44).

- Use 10 ng DNA in 10 µL reactions on 384-well plates.
- Use 20 ng DNA in 10 µL reactions on 96-well plates.

IMPORTANT! You must use the same amount of gDNA for each sample and for each sample replicate that is run with the same assay.

Experimental setup conditions

Sample types to be run on each plate

- **Samples or Unknowns –** gDNA samples in which the copy number of the target is unknown.
- **No Template Control (NTC) –** A sample that does not contain a DNA template. It shows the background fluorescence and allows for the detection of contamination.
- **Calibrator sample –** A DNA sample with a known copy number for the target of interest. Also known as the reference sample.

Template replicates

To generate the reliable copy number calls, Life Technologies strongly recommends using four replicates for each gDNA sample.

Number of samples run per plate

If CopyCaller® Software is used for copy number analysis (recommended), quality metrics including confidence values will be calculated when at least 7 samples of the same copy number group are present on the plate. If fewer than 7 samples of the same copy number are present, copy number values will be calculated, but quality metrics will not be calculated.

Set up the instrument software

Use your QuantStudio® 12K Flex Software to set up the experiment. Use the Standard Curve experiment type to capture the cycle threshold (C_T) data from the duplex PCR run. The real-time PCR C_T data are subsequently used by CopyCaller® Software to calculate sample copy number values by relative quantitation.

Create a 384-well or 96-well plate experiment for the run using the Standard Curve experiment type, TaqMan® Reagents, and the Standard run mode.

Define Target names:

- Enter the TaqMan® Copy Number Assay ID or user-defined name as the Target Name with FAM as the Reporter and NFQ-MGB as the Quencher.

  If using AlleleTyper™ Software for data analysis, the copy number assay id must contain the suffix "_cn" and must match the target assay name used in the translation table.
- Enter the TaqMan® Copy Number Reference Assay gene target or user-defined name as the Target Name with VIC® as the Reporter and TAMRA as the Quencher.

Assign to each well of the plate that contains a reaction: a sample name and a detector/target that includes dye information (reporter and quencher).

Create unique sample names so that the CopyCaller® Software analyzes each sample separately. Apply the same sample name to the wells of each technical replicate group.

Prepare the reactions

1. Calculate the volumes of components that you need, based on the reaction volume and the number of reactions. Include excess volume in your calculations to provide for the loss that occurs during reagent transfers.

   Note: Life Technologies recommends using *four replicates* of each sample.

Reaction mixture component	Volume per well	
	384-well plate	96-well plate
2X TaqMan® Genotyping Master Mix†	5.0 µL	10.0 µL
TaqMan® Copy Number Assay, 20X working stock‡	0.5 µL	1.0 µL
TaqMan® Copy Number Reference Assay, 20X	0.5 µL	1.0 µL
Nuclease-free water	2.0 µL	4.0 µL
Total Volume	**8.0 µL**	**16.0 µL**

† TaqMan® Gene Expression or TaqMan® Universal Master Mixes can also be used, but *do not* use TaqMan® Fast Universal Master Mix.
‡ If you use large-scale assays (60X), dilute the assays to a 20X working stock.

2. Completely thaw the TaqMan® Copy Number Assays and the TaqMan® Copy Number Reference Assays. Gently vortex the assays to mix them, then centrifuge the tubes briefly to bring contents to the bottom of the tube.
3. Swirl to thoroughly mix the TaqMan® Genotyping Master Mix.

4. Combine the required volumes of reaction components in microcentrifuge tubes.
5. Invert or flick the tubes to mix the contents thoroughly, then centrifuge the tubes briefly.
6. Pipette the reaction mixture into the wells of the reaction plate that you prepared.
   - For 384-well plates – pipette 8 µL per well.
   - For 96-well plates – pipette 16 µL per well.
7. Vortex the gDNA samples that you prepared and diluted.
8. Add the gDNA to the wells containing the reaction mixture:
   - For 384-well plates – pipette 2 µL of gDNA (5 ng/µL) per well.
   - For 96-well plates – pipette 4 µL of gDNA (5 ng/µL) per well.

   Note: Alternatively, you can add the gDNA to the plate first, then add the reaction mixture.
9. Mix the reaction mixture with the gDNA by pipetting up and down several times.
10. Seal the reaction plate with optical adhesive film (or optical caps), then centrifuge the reaction plate briefly.
11. Inspect all the wells to ensure a uniform volume.
12. Proceed to the next step, "Run the reactions."

Run the reactions

Run the plate

1. Load the reaction plate into a real-time PCR instrument.
2. Run the plate using the parameters below:

Stage	Temperature	Time
Hold	95 °C	10 min
Cycle	95 °C	15 sec
(40 Cycles)	60 °C	60 sec

3. Unload the reaction plate after the run is complete.

Analyze and export results

Analyze the results

1. In the real-time PCR Instrument software, open the Analysis Settings window and set the following:
   - Manual C_T threshold – **0.2**
   - Autobaseline – **On**
2. Apply the settings, then close the window.
3. Analyze the experiment.

4. Review the analyzed data and troubleshoot any flags or problematic data. Verify that the amplification curves for the:
   - Reference Assay (VIC® dye signal) in all samples have a distinct, linear amplification phase.
   - Copy Number Assay (FAM™ dye signal) in most wells have a distinct, linear amplification phase.

     Note: Samples that contain zero copies of the target of interest do not amplify well, if at all, with the copy number assay. Such samples have high or undetermined FAM™ C_Ts.
   - Review any displayed quality check (QC) flags, then review the real-time data of the associated samples.

For information on troubleshooting problematic real-time data, see Appendix C, "Troubleshooting" on page 98 or refer to the User Guide or Getting Started Guides for your Real-Time PCR System.

Export the results

After you use the Real-Time PCR System software to analyze each TaqMan® Copy Number Assay experiment, export experiment results to one or more exported real-time PCR files. Use the tab-delimited (.txt) format if using CopyCaller® Software for downstream copy number analysis (recommended).

Analyze results using CopyCaller® Software

About CopyCaller® Software

CopyCaller® Software performs relative quantitation analysis of genomic DNA targets using the real-time PCR data from TaqMan® Copy Number Assay experiments run on Applied Biosystems® Real Time PCR Systems. including QuantStudio® 12K Flex System. The software and associated copy number assays can be used to detect and measure copy number variation of specific genomic sequences. Use CopyCaller® Software to analyze the real-time PCR data and to export copy number data files that you can import into AlleleTyper™ Software for translation.

CopyCaller® Software performs a comparative C_T ($\Delta\Delta C_T$) relative quantitation analysis of the real-time data. The analysis determines the number of copies of the target sequence in each test genomic DNA sample. The comparative CT ($\Delta\Delta C_T$) method first calculates the difference (ΔC_T) between the threshold cycles of the target and reference assay sequences. Then, the method compares the ΔC_T values of the test samples to a calibrator sample that contains a known number of copies of the target sequence. Alternatively, the analysis can be performed without the use of a calibrator sample by using a maximum likelihood algorithm.

Note: For a complete description of CopyCaller® Software features and guidance on using the software, refer to the *CopyCaller® Software v2.0 User Guide* (Pub. no. 4400042)

Perform copy number analysis

Import the results files

1. Start CopyCaller® Software.
2. In the CopyCaller® Software toolbar, click  (Import real-time PCR results file) or select **File ▸ Impor**t.
3. In the Import dialog box, select one or more real-time PCR files to analyze.

4. Click **Open** to import the data from the selected real-time PCR results.
5. Repeat steps through as needed to add files to the analysis.

Select and analyze assay data

1. In the Assay Selection Table, select one or more assays to analyze. To select an assay, click anywhere in a row of the Assay Selection Table (the software highlights the selected row in blue).
2. In the toolbar, click (Analysis Settings).
3. In the Analysis Settings dialog box Calibrator selection panel, specify the calibrator sample settings for the selected assay(s) depending on whether or not a calibrator sample of known copy number is available.

   If a calibrator is present:

   a. Select **With Calibrator Sample**.
   b. In the Calibrator Sample Name drop-down list, select or enter a sample to use as the calibrator for the analysis. The median ΔC_T value is also available can also be selected as the calibrator and works best when most samples have an equivalent copy number.
   c. In the Calibrator Sample Copy Number field, enter the number of copies of the target sequence that are in the calibrator sample. The number of copies must be a whole number greater than zero.

   If a calibrator is not present:

   a. Select **Without Calibrator Sample**.
   b. In the Most Frequent Sample Copy Number field, enter the number of copies of the target sequence expected in the majority of samples. The number of copies must be a whole number greater than zero.
4. *(Optional*) Expand the Advanced settings box to review and/or edit empirical thresholds, or to create copy number bins for confidence estimates: Refer to the *CopyCaller® Software v2.0 User Guide* (Pub. no. 4400042) for more information on these features.
5. Click **Apply** to apply the analysis settings and perform copy number analysis using the revised analysis settings
6. Repeat steps through as necessary to analyze any remaining assays.
7. Display the results of the analysis:
   a. In the Assay Selection Table, verify that the assays that you want to add to the analysis show "Y" in the (Analysis Status) column. If not, analyze the unanalyzed assay(s) as explained above.
   b. In the (Display Analysis Results) column, select the check boxes for up to 10 analyzed assays that you want to display.

Review results

Review the Copy Number Plot

- Samples should have calculated copy number values close to integers and small range bars.
- Review the plot for intermediate copy numbers. The presence of intermediate, calculated copy number values (such as 1.5) can indicate that the calibrator or copy number was specified incorrectly, or a potential problem exists with the associated test sample or calibrator sample.
- Review the copy number range bars for each sample. Large bars may indicate that the technical replicates of the associated sample exhibit a broad range of ΔC_T values, possibly indicating that sample data quality is suboptimal.

  Note: The copy number range of replicates is frequently larger for samples that have high target copy numbers (>3) as a result of their smaller ΔC_T values.

Review the Results Table

- Examine the confidence values and absolute z-score values to assess the reliability of each copy number call.
- Review samples having a predicted copy number of "Undetermined," which occurs if the reference assay did not amplify sufficiently, indicating low sample quantity or quality.

  A sample is "Undetermined" if CopyCaller® Software cannot analyze the sample because the:
  - Reference assay did not amplify sufficiently, possibly indicating low sample quality.
  - Replicate data for a sample were conflicting.
- Review samples having a predicted copy number of 0 (zero-copy samples).

  Zero-copy samples produce reference assay amplification (passing VIC® dye) and weak or nonexistent target amplification.

  CopyCaller® Software cannot calculate confidence values for zero-copy-number samples. However, samples that produce no FAM™ signal are, by definition, high-confidence calls because no target DNA was amplified.
- Review samples having a predicted copy number ≥1.

  CopyCaller® Software calculates confidence and absolute z-score values for each sample set that has a non-zero predicted copy number value and sufficient data for the estimation.

  Note: The software cannot calculate the confidence and absolute z-score values for sample sets that have fewer than seven samples of a single copy number because the algorithm requires a minimum number of data points.

- Under optimal experimental conditions where samples are of high quality, copy number and reference assays have amplified, and sample replicates have similar C_T and ΔC_T values:
  - Samples that have low copy numbers (1, 2, or 3) commonly have confidence values greater than 95%.
  - As copy numbers increase, confidence progressively decreases due to the decreased separation of ΔC_T subdistribution values of copy numbers.
  - Review samples that have confidence values greater than 95%.

    Samples that have high confidence values can sometimes deviate significantly from the mean copy number for the copy number subdistribution. For sample copy number calls with confidence values greater than 95%, look at the absolute z-scores, then consider accepting or rejecting the copy number call based on the following:

|Z-Score|	Status
< 1.75	Pass
2.65 > z ≥ 1.75	Pass with caution
≥ 2.65	Fail

Note: The thresholds in the table above are based on empirical observations and are provided only as guidelines.

(*Optional*) Edit the analysis settings

1. Select one or more assays in the Assay Selection Table, then click **View Analysis Settings** in the toolbar.
2. Revise the analysis settings as needed.

   For example, if the quality of the calibrator data is poor, you can select a different calibrator sample and reanalyze the data
3. Click **Apply** to perform the copy number analysis using the revised analysis settings.

Review the Well Table

For each replicate group in the Well Table, review the Flag column for any quality flags generated during the analysis, and determine the source of the warning.

Some quality flags indicate potential issues with wells or samples. For example, wells that generate NOVIC or VICET flags did not amplify the reference assay target properly and may contain low-quantity or poor-quality DNA.

Review the Analysis Summary

Review the Analysis Summary tab for the summarized results of the copy number analysis. While viewing the summary, you can copy and paste the data into other applications.

Review the Statistics Chart

Review the Statistics Chart tab for information about the distribution of copy numbers among the samples in a copy number assay. While viewing the Statistics Chart, you can copy, save, or print it.

Review the ΔC_T Plot

Review the ΔC_T Plot tab for information about the distribution of sample ΔC_T values in a copy number assay experiment. While viewing the ΔC_T Plot, you can copy, save, or print it.

7 Perform translation analysis in AlleleTyper™ Software

This chapter covers:

Introduction to AlleleTyper™ Software

AlleleTyper™ Software is an automated data analysis application that translates genetic pattern information from TaqMan® SNP Genotyping Assay and/or TaqMan® Copy Number Assay results to user-defined genotype or diplotype nomenclature. For complete instructions on how to use AlleleTyper™ Software and to create user-defined translation tables, please refer to the *AlleleTyper™ Software User Guide* (Pub. no. 4486002).

AlleleTyper™ Software is a flexible tool used for conversion of sample genotype information for single or multiple genes or loci to the desired nomenclature, for example, the PGx gene-level star (*) allele nomenclature to describe SNP, InDel or copy number variant genotypes (e.g., for the Cytochrome P450 genes). Star alleles are haplotypes that can contain multiple variants; these are associated with functional or nonfunctional gene products. AlleleTyper™ Software can also be used to translate the genotype results for special cases including triallelic or adjacent SNP interrogation using two TaqMan® SNP assays, as well as used to simply provide a name for a particular genetic outcome for a given SNP or copy number assay.

Before creating a translation table, or translator, consider all TaqMan® SNP Genotyping Assays (includes TaqMan® DME Assays) and TaqMan® Copy Number Assays that will be used in your project, and which of these you will want to include in a translation table for reporting sample genotype results using specific nomenclature. SNP assay and copy number assay experiment data generated on an Applied Biosystems® real-time PCR system must be analyzed by TaqMan® Genotyper™ Software and CopyCaller® Software, respectively, because results files exported from these software systems are input files for AlleleTyper™ Software.

AlleleTyper™ Software aids creation of translation tables by automatically converting monoallelic translation tables containing haplotype patterns to biallelic translators containing diplotype or genotype patterns. After examining biallelic translators for completeness and accuracy, they are imported into AlleleTyper™ Software along with TaqMan® Genotyper™ Software data files and/or CopyCaller® Software data files. AlleleTyper™ Software then matches the sample genetic pattern data to patterns in the biallelic translator and reports back the designated nomenclature for each matching pattern, on a gene-by-gene basis when a multigene translator is used.

Sources of PGx translation information and example translation tables

Life Technologies provides example AlleleTyper™ Software translation table template files and translation tables (including monoallelic and biallelic tables; single gene and multigene translators) for commonly tested drug metabolism gene variants. These can be downloaded from the AlleleTyper™ Software web page on **www.lifetechnologies.com/pgx** and used as templates for creating translators specific to your TaqMan® Assays panel.

The PharmGKB website (**www.pharmgkb.org**) provides comprehensive haplotype translation tables for CYP gene variants found in the Human Cytochrome P450 (CYP) Allele Nomenclature Database (**www.cypalleles.ki.se**) as well as for other drug metabolism gene variants. These can be used as reference tables for creating AlleleTyper™ Software translation tables.

The translation tables provided by both Life Technologies and PharmGKB use the convention whereby the variant alleles are provided in the (+) strand orientation of the reference genome. Likewise, TaqMan® SNP and DME Genotyping Assays context sequences are provided in the (+) genome strand orientation. In contrast, the * allele nomenclature for many PGx genes does not follow this convention: the variant alleles may map to the (–) strand of the reference genome and thus are given in the reverse complement orientation (e.g., CYP2D6, which maps to the (–) genome strand). For successful translation of sample genotyping results by AlleleTyper™ Software, the base alleles used in your translation table must match the base alleles used in the TaqMan® Genotyper™ Software results files. As well, the assay identifiers must match between the translation table and the TaqMan® Genotyper™ and/or CopyCaller® Software results files.

A DNA isolation using the MagMAX™ DNA Multi-Sample Ultra Kit

Refer to the *MagMAX™ DNA Multi-Sample Ultra Kit User Guides* on page 80 (buccal swab) or page 84 (whole blood) for detailed procedures for isolating and purifying genomic DNA.

These user guides are also available at the product web page at **www.lifetechnologies.com**.

USER GUIDE

MagMAX™ DNA Multi-Sample Ultra Kit

High-throughput isolation of PCR-ready DNA from buccal swabs

Catalog Number A25597 and A25598

Pub. No. MAN0010293 **Rev.** C.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

Product information

The MagMAX™ DNA Multi-Sample Ultra Kit is designed for rapid, high-throughput isolation of high-quality genomic DNA from a variety of sample matrices. The kit uses MagMAX™ magnetic bead technology, ensuring reproducible recovery of PCR-ready DNA suitable for a broad range of applications, such as SNP genotyping and copy number experiments.

This protocol describes isolation of DNA from buccal swabs, optimized for use with the MagMAX™ Express-96 Deep Well Magnetic Particle Processor or with the KingFisher™ Flex Magnetic Particle Processor (96-well deep well setting). The typical DNA yield obtained from 1 buccal swab is 2–12 µg at a concentration suitable for OpenArray® analysis.

Kit contents and storage

Component	Cat. no. A25597[1] (500 rxns)	Cat. no. A25598[2] (2500 rxns)	Storage
Proteinase K[3]	4 mL	5 × 4 mL	–15°C to –25°C
PK Buffer	96 mL	5 × 96 mL	15°C to 30°C
Multi-Sample DNA Lysis Buffer	100 mL	5 × 100 mL	
RNase A[4]	2 × 1.25 mL	10 × 1.25 mL	–15°C to –25°C
DNA Binding Beads[3]	8 mL	5 × 8 mL	2°C to 8°C
Nuclease-free Water	100 mL	5 × 100 mL	15°C to 30°C
Wash Solution 1 Concentrate	80 mL[5]	5 × 80 mL[5]	
Wash Solution 2 Concentrate	162 mL[5]	5 × 162 mL[5]	
DNA Elution Buffer 1	25 mL	5 × 25 mL	
DNA Elution Buffer 2	25 mL	5 × 25 mL	

[1] Also available as Cat. no. A25919, containing Cat. no. A25597 with **one additional tube** each of Proteinase K (4 mL) and DNA Binding Beads (8 mL).

[2] Also available as Cat. no. A25920, containing Cat. no. A25598 with **5 additional tubes** of Proteinase K (4 mL each) and **5 additional tubes** of DNA Binding Beads (8 mL each).

[3] Proteinase K is also available as Cat. no. A25561 and DNA Binding Beads are also available as Cat. no. A25562.

[4] Not used for DNA isolation from buccal swabs.

[5] Final volume; see "Before first use of the kit: prepare Wash Solutions" on page 2.

Materials required but not supplied

Unless otherwise specified, all materials are available from Life Technologies. MLS: major laboratory supplier.

Item	Source
Magnetic particle processor	
MagMAX™ Express-96 Magnetic Particle Processor	Cat. no. 4400077
KingFisher™ Flex Magnetic Particle Processor[1]	Thermo Scientific Cat. no. 5800630
Equipment	
Thermo Scientific™ Titer Plate Shaker	Cat. no. 14-271-9[2]
One of the following incubators, or an equivalent incubator with slatted shelves and thermometer:	
Economy Lab Incubator	Cat. no. S50441A[2]
VWR® Digital Mini Incubator	VWR Cat. no. 10055-006
Fisher Scientific™ Analog Vortex Mixer	Cat. no. 02-215-365[2]
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
(Optional but recommended) Magnetic Stand-96	Cat. no. AM10027
Plastics and consumables	
MagMAX™ Express-96 Deep Well Plates	Cat. no. 4388476
MagMAX™ Express-96 Standard Plates	Cat. no. 4388475
MagMAX™ Express-96 Deep Well Tip Combs	Cat. no. 4388487
MicroAmp® Clear Adhesive Film	Cat. no. 4306311
Puritan™ PurFlock™ Ultra Flocked Swabs	Cat. no. 22-025-192[2]
RNase-free Microfuge Tubes (2.0 mL)	Cat. no. AM12425
Conical tubes (15 mL)	Cat. no. AM12500
Conical tubes (50 mL)	Cat. no. AM12502
Aerosol-resistant pipette tips	MLS
Reagent reservoirs	MLS
Reagents	
Ethanol, 200 proof (absolute)	MLS
Isopropanol, 100% (molecular grade or higher)	MLS
(Optional) Proteinase K, 500 reactions (4 mL)	Cat. no. A25561
(Optional) DNA Binding Beads, 500 reactions (8 mL)	Cat. no. A25562

[1] See "If needed, download the KingFisher™ Flex program" on page 2.

[2] Available from Fisher Scientific

For Research Use Only. Not for use in diagnostic procedures.

Sample collection and storage

Test subjects should thoroughly rinse their mouths with water and swallow prior to swabbing.

1. Remove swab from container and thoroughly swab both cheeks of the test subject for 30 seconds each to maximize collection of buccal cells.

   Note: Use polyester swabs for optimum results (cotton swabs may contain PCR inhibitors). We recommend using Puritan™ PurFlock™ Ultra Flocked Swabs (Fisher Scientific; Cat. no. 22-025-192).
2. Process or store samples shortly after collection. Alternatively, they can be stored:
   - At room temperature or 4°C overnight.
   - Between –20°C and –80°C for up to several weeks.

Important procedural guidelines

- Perform all steps at room temperature (20–25°C) unless otherwise noted.
- Equilibrate buccal swabs to room temperature to maximize DNA recovery.
- Cover the plate during incubation and shaking steps to prevent spill-over and cross-contamination. The same MicroAmp® Clear Adhesive Film can be used throughout the procedure, unless it becomes contaminated.
- If you use a plate shaker other than the Thermo Scientific™ Titer Plate Shaker , verify that:
  – The MagMAX™ Express-96 Deep Well Plate fits securely on your titer plate shaker.
  – The recommended speeds are compatible with your titer plate shaker. Ideal speeds should allow for thorough mixing without splashing.
- Per-plate volumes for reagent mixes are sufficient for one 96-well plate plus overage. To calculate volumes for other sample numbers, refer to the per-well volume and add 5% overage.
- If the DNA yield is lower than expected, extend the Proteinase K digestion time to 45 minutes.

If needed, download the KingFisher™ Flex program

The program required for this protocol is not pre-installed on the KingFisher™ Flex Magnetic Particle Processor.

1. On the MagMAX™ DNA Multi-Sample Ultra Kit web page, scroll down to the **Product Literature** section.
2. Right-click **A25597_Blood_Buccal** and select **Save as Target** to download to your computer.
3. Refer to *Thermo Scientific™ KingFisher™ Flex User Manual* (Cat. no. N07669) and *BindIt™ Software User Manual* (Cat. no. N07974) for instructions for installing the program on the instrument.

Before first use of the kit: prepare Wash Solutions

Prepare the Wash Solutions from the concentrates:

- Add 25 mL of isopropanol to Wash Solution 1 Concentrate, mix, and store at room temperature.
- Add 132 mL of ethanol to Wash Solution 2 Concentrate, mix, and store at room temperature.

Before each use of the kit: prepare DNA Binding Bead Mix

Vortex DNA Binding Beads thoroughly before pipetting and combine with Nuclease-free Water according to the following table.

Component	Volume per well	Volume per plate
DNA Binding Beads	16 µL	1.6 mL
Nuclease-free Water	24 µL	2.4 mL
Total DNA Binding Bead Mix	40 µL	4 mL

Store DNA Binding Bead Mix at room temperature for no longer than 24 hours.

Perform DNA extraction and elution

1 Digest the samples with Proteinase K

a. Preheat an incubator to 65°C.
b. Place the swabs in the wells of a MagMAX™ Express-96 Deep Well Plate (one per well).
c. Break the stick off the swabs enough to sit in the well without protruding.
d. Prepare sufficient PK Mix according to the following table.

IMPORTANT! Prepare the PK Mix just before use. Do not place PK Buffer or PK Mix on ice to avoid precipitation.

Component	Volume per well	Volume per plate
Proteinase K	8 µL	800 µL
PK Buffer	192 µL	19.2 mL
Total PK Mix	200 µL	20 mL

e. Invert PK Mix several times to thoroughly mix components, then add 200 µL to each well containing a swab.

IMPORTANT! Do not touch the plastic base of the swab with the pipet tips when pipetting the PK Mix in the sample wells.

f. Seal the plate with a MicroAmp® Clear Adhesive Film.
g. Shake the sealed plate for 5 minutes at speed 8.
h. Incubate for 20–45 minutes at 65°C.

IMPORTANT! Arrange samples in the incubator to allow adequate flow around the plate wells, to ensure that samples quickly reach and maintain the incubation temperature.

2 Set up the MagMAX™ Express-96 processing plates

While the samples are incubating at 65°C, set up the MagMAX™ Express-96 Wash, Elution, and Tip Comb Plates outside the instrument as described in the following table.

Table 1 MagMAX™ Express- 96 processing plates

Plate ID	Plate position[1]	MagMAX™ Express-96 plate type	Reagents	Volume per well
Wash Plate 1	2	Deep Well	Wash Solution 1	150 µL
Wash Plate 2	3	Deep Well	Wash Solution 2	150 µL
Wash Plate 3	4	Deep Well	Wash Solution 2	150 µL
Elution Plate[2]	5	Standard	DNA Elution Buffer 1	50 µL
Tip Comb	6	Deep Well	Place a MagMAX™ Express-96 Deep Well Tip Comb in a MagMAX™ Express-96 Deep Well Plate.	

[1] Position on the instrument

[2] The instrument prompts the user to add DNA Elution Buffer 2 to the Elution Plate, after incubation with DNA Elution Buffer 1 ("Wash the beads and elute the DNA" on page 3).

3 Add Multi-Sample DNA Lysis Buffer, isopropanol, and DNA Binding Bead Mix

a. (Optional) At the end of the 65°C incubation, briefly centrifuge the plate for 1–2 minutes at 1500 × *g* if condensation is present.
b. Add 200 µL of Multi-Sample DNA Lysis Buffer to each sample.
c. Seal the plate with a MicroAmp® Clear Adhesive Film and shake for 5 minutes at speed 8.
d. Transfer lysates to the wells of a new MagMAX™ Express-96 Deep Well Plate (PK Plate) and discard the plate containing the buccal swabs.
e. Add 240 µL of isopropanol to each sample, seal the plate, and shake for 5 minutes at speed 8.
f. Vortex DNA Binding Bead Mix at moderate speed to ensure thorough mixing, add 40 µL to each sample, and proceed immediately to DNA isolation (next step).

4 Wash the beads and elute the DNA

a. Select the program on the instrument.
   - **4413021 DW blood** on MagMAX™ Express-96 Magnetic Particle Processor
   - **A25597_Blood_Buccal** on KingFisher™ Flex Magnetic Particle Processor
b. Start the run and load the prepared processing plates to their positions when prompted by the instrument (see Table 1).
c. Load the PK Plate (containing lysate, isopropanol, and DNA Binding Bead Mix) at position 1 when prompted by the instrument .
d. When prompted by the instrument (approximately 28–30 minutes into the run):
   1. Remove the Elution Plate from the instrument.
   2. Add 50 µL of DNA Elution Buffer 2 to each sample well.

   IMPORTANT! Add DNA Elution Buffer 2 immediately after the prompt to prevent excessive drying of any beads that are still captured on the Tip Comb.

   3. Load the Elution Plate back onto the instrument, and press **Start**.
e. At the end of the run (approximately 30–35 minutes after initial start), remove the Elution Plate from the instrument and seal immediately with a new MicroAmp® Clear Adhesive Film.
   - If precipitated DNA is visible, pipet up and down 5–10 times before sealing the plate, to ensure complete resuspension.
   - Eluates can also be transferred to a new elution plate after collection.
   - If excess bead residue is seen in the wells, place the Elution Plate on the Magnetic Stand-96 to collect any residue prior to downstream use of the DNA.

   IMPORTANT! Do not allow the purified samples to sit uncovered at room temperature for more than 10 minutes, to prevent evaporation and contamination.

The purified samples are ready for immediate use. Alternatively, store the covered Elution Plate:
- At 2–6°C for up to 24 hours.
- At –20°C or –80°C for long-term storage.

Recommended quantitation methods

Standard curve analysis is the most accurate quantitation method whereas UV absorbance measurements can be used to assess both the concentration and the quality of the isolated DNA.

- **Standard curve analysis.** Use the TaqMan® RNase P Copy Number Reference Assay (Cat. no. 4403326) for human genomic DNA and the TaqMan® DNA Template Reagents (Cat. no. 401970) to create a standard curve. Refer to *Creating Standard Curves with Genomic DNA or Plasmid DNA Templates for Use in Quantitative PCR* (Pub. no. 4371090).
- **UV absorbance measurements.** Use a NanoDrop® or other comparable instrument. Pure genomic DNA should have an A_{260}/A_{280} ratio of approximately 1.6–2.0.

Note: Mix the samples by pipetting up and down before quantitation if they have been stored frozen.

Revision history

Revision	Date	Description
C.0	July 2014	Addition of important procedural guidelines
B.0	July 2014	Correction of the kit name
A.0	May 2014	New document

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

USER GUIDE

MagMAX™ DNA Multi-Sample Ultra Kit

High-throughput isolation of PCR-ready DNA from whole blood

Catalog Number A25597 and A25598

Pub. No. MAN0010294 **Rev.** C.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **www.lifetechnologies.com/support**.

Product information

The MagMAX™ DNA Multi-Sample Ultra Kit is designed for rapid, high-throughput isolation of high-quality genomic DNA from a variety of sample matrices. The kit uses MagMAX™ magnetic bead technology, ensuring reproducible recovery of PCR-ready DNA suitable for a broad range of applications, such as SNP genotyping and copy number experiments.

This protocol describes isolation of DNA from mammalian whole blood samples, optimized for use with the MagMAX™ Express-96 Deep Well Magnetic Particle Processor or with the KingFisher™ Flex Magnetic Particle Processor (96-well deep well setting). The typical DNA yield obtained from 50 µL of whole blood is 1.5–4 µg at a concentration suitable for OpenArray® analysis.

Kit contents and storage

Component	Cat. no. A25597[1] (500 rxns)	Cat. no. A25598[2] (2500 rxns)	Storage
Proteinase K[3]	4 mL	5 × 4 mL	–15°C to –25°C
PK Buffer	96 mL	5 × 96 mL	15°C to 30°C
Multi-Sample DNA Lysis Buffer	100 mL	5 × 100 mL	
RNase A	2 × 1.25 mL	10 × 1.25 mL	–15°C to –25°C
DNA Binding Beads[3]	8 mL	5 × 8 mL	2°C to 8°C
Nuclease-free Water	100 mL	5 × 100 mL	15°C to 30°C
Wash Solution 1 Concentrate	80 mL[4]	5 × 80 mL[4]	
Wash Solution 2 Concentrate	162 mL[4]	5 × 162 mL[4]	
DNA Elution Buffer 1	25 mL	5 × 25 mL	
DNA Elution Buffer 2	25 mL	5 × 25 mL	

[1] Also available as Cat. no. A25919, containing Cat. no. A25597 with **one additional tube** each of Proteinase K (4 mL) and DNA Binding Beads (8 mL).

[2] Also available as Cat. no. A25920, containing Cat. no. A25598 with **5 additional tubes** of Proteinase K (4 mL each) and **5 additional tubes** of DNA Binding Beads (8 mL each).

[3] Proteinase K is also available as Cat. no. A25561 and DNA Binding Beads are also available as Cat. no. A25562.

[4] Final volume; see "Before first use of the kit: prepare Wash Solutions" on page 2.

Materials required but not supplied

Unless otherwise specified, all materials are available from Life Technologies. MLS: major laboratory supplier.

Item	Source
Magnetic particle processor	
MagMAX™ Express-96 Magnetic Particle Processor	Cat. no. 4400077
KingFisher™ Flex Magnetic Particle Processor[1]	Thermo Scientific Cat. no. 5800630
Equipment	
Thermo Scientific™ Titer Plate Shaker	Cat. no. 14-271-9[2]
One of the following incubators, or an equivalent incubator with slatted shelves and thermometer:	
Economy Lab Incubator	Cat. no. S50441A[2]
VWR® Digital Mini Incubator	VWR Cat. no. 10055-006
Fisher Scientific™ Analog Vortex Mixer	Cat. no. 02-215-365[2]
Adjustable micropipettors	MLS
Multi-channel micropipettors	MLS
(Optional but recommended) Magnetic Stand-96	Cat. no. AM10027
Plastics and consumables	
MagMAX™ Express-96 Deep Well Plates	Cat. no. 4388476
MagMAX™ Express-96 Standard Plates	Cat. no. 4388475
MagMAX™ Express-96 Deep Well Tip Combs	Cat. no. 4388487
MicroAmp® Clear Adhesive Film	Cat. no. 4306311
RNase-free Microfuge Tubes (2.0 mL)	Cat. no. AM12425
Conical tubes (15 mL)	Cat. no. AM12500
Conical tubes (50 mL)	Cat. no. AM12502
Aerosol-resistant pipette tips	MLS
Reagent reservoirs	MLS
Reagents	
Ethanol, 200 proof (absolute)	MLS
Isopropanol, 100% (molecular grade or higher)	MLS
(Optional) Proteinase K, 500 reactions (4 mL)	Cat. no. A25561
(Optional) DNA Binding Beads, 500 reactions (8 mL)	Cat. no. A25562

[1] See "If needed, download the KingFisher Flex° program" on page 2.

[2] Available from Fisher Scientific

For Research Use Only. Not for use in diagnostic procedures.

Sample collection and storage

1. Collect blood samples using proper venipuncture collection and handling procedures in EDTA or sodium citrate anticoagulant tubes.

   Note: Heparin is not recommended as an anti-coagulant since it can cause inhibition of PCR reactions.
2. Invert the tube to ensure thorough mixing.
3. Process samples shortly after collection or freeze them immediately after collection and store them between –20°C and –80°C.

   IMPORTANT! To minimize DNA degradation, do not re-freeze samples after they have been thawed. We recommend storing the blood in single-use aliquots to avoid multiple freeze/thaw cycles.

Important procedural guidelines

- If the whole blood is frozen prior to use, thaw the sample at 25–37°C in a water bath until it is completely liquid, and place on ice until needed.
- Perform all steps at room temperature (20–25°C) unless otherwise noted.
- When mixing samples by pipetting up and down, avoid creating bubbles.
- Cover the plate during incubation and shaking steps to prevent spill-over and cross-contamination. The same MicroAmp® Clear Adhesive Film can be used throughout the procedure, unless it becomes contaminated.
- If you use a plate shaker other than the Thermo Scientific™ Titer Plate Shaker , verify that:
  - The MagMAX™ Express-96 Deep Well Plate fits securely on your titer plate shaker.
  - The recommended speeds are compatible with your titer plate shaker. Ideal speeds should allow for thorough mixing without splashing.
- Per-plate volumes for reagent mixes are sufficient for one 96-well plate plus overage. To calculate volumes for other sample numbers, refer to the per-well volume and add 5% overage.
- If the DNA yield is lower than expected, extend the Proteinase K digestion time to 45 minutes.

If needed, download the KingFisher™ Flex program

The program required for this protocol is not pre-installed on the KingFisher™ Flex Magnetic Particle Processor.

1. On the MagMAX™ DNA Multi-Sample Ultra Kit web page, scroll down to the **Product Literature** section.
2. Right-click **A25597_Blood_Buccal** and select **Save as Target** to download to your computer.
3. Refer to *Thermo Scientific™ KingFisher™ Flex User Manual* (Cat. no. N07669) and *BindIt™ Software User Manual* (Cat. no. N07974) for instructions for installing the program on the instrument.

Before first use of the kit: prepare Wash Solutions

Prepare the Wash Solutions from the concentrates:

- Add 25 mL of isopropanol to Wash Solution 1 Concentrate, mix, and store at room temperature.
- Add 132 mL of ethanol to Wash Solution 2 Concentrate, mix, and store at room temperature.

Perform DNA extraction and elution

1 Digest the samples with Proteinase K

a. Preheat an incubator to 65°C.
b. Prepare sufficient PK Mix according to the following table.

IMPORTANT! Prepare the PK Mix just before use. Do not place PK Buffer or PK Mix on ice to avoid precipitation.

Component	Volume per well	Volume per plate
Proteinase K	8 µL	800 µL
PK Buffer	192 µL	19.2 mL
Total PK Mix	200 µL	20 mL

c. Invert PK Mix several times to thoroughly mix components, then add 200 µL to each sample well of a MagMAX™ Express-96 Deep Well Plate (PK Plate).
d. Transfer 50 µL of whole blood to the appropriate well containing PK Mix.

IMPORTANT! Invert the tube containing the blood sample before pipetting to ensure homogenous mixing.

e. Seal the plate with a MicroAmp® Clear Adhesive Film.
f. Shake the sealed plate for 5 minutes at speed 8.
g. Incubate for 20–45 minutes at 65°C.

IMPORTANT! Arrange samples in the incubator to allow adequate flow around the plate wells, to ensure that samples quickly reach and maintain the incubation temperature.

2 Set up the MagMAX™ Express-96 processing plates

While the samples are incubating at 65°C, set up the MagMAX™ Express-96 Wash, Elution, and Tip Comb Plates outside the instrument as described in the following table.

Table 1 MagMAX™ Express- 96 processing plates

Plate ID	Plate position[1]	MagMAX™ Express-96 plate type	Reagents	Volume per well
Wash Plate 1	2	Deep Well	Wash Solution 1	150 µL
Wash Plate 2	3	Deep Well	Wash Solution 2	150 µL
Wash Plate 3	4	Deep Well	Wash Solution 2	150 µL
Elution Plate[2]	5	Standard	DNA Elution Buffer 1	50 µL
Tip Comb	6	Deep Well	Place a MagMAX™ Express-96 Deep Well Tip Comb in a MagMAX™ Express-96 Deep Well Plate.	

[1] Position on the instrument

[2] The instrument prompts the user to add DNA Elution Buffer 2 to the Elution Plate, after incubation with DNA Elution Buffer 1 ("Wash the beads and elute the DNA" on page 3).

3 Add Multi-Sample DNA Lysis Buffer, Bead/RNase Mix, and isopropanol

a. (Optional) At the end of the 65°C incubation, briefly centrifuge the PK Plate for 1–2 minutes at 1500 × *g* if condensation is present.
b. Add 200 µL of Multi-Sample DNA Lysis Buffer to each sample.
c. Seal the PK Plate with the MicroAmp® Clear Adhesive Film and shake for 5 minutes at speed 8.
d. Prepare Bead/RNase Mix according to the following table.

IMPORTANT! Prepare the Bead/RNase Mix up to 20 minutes before use. Prolonged storage at room temperature can reduce its efficiency. Vortex the DNA Binding beads at moderate speed to form a uniform suspension before pipetting.

Component	Volume per well	Volume per plate
DNA Binding Beads	16 µL	1.6 mL
RNase A	5 µL	500 µL
Nuclease-free Water	19 µL	1.9 mL
Total Bead/RNase Mix	40 µL	4 mL

e. Vortex the Bead/RNase Mix at moderate speed to ensure thorough mixing, then add 40 µL to each sample and pipet up and down 5 times using a multi-channel micropipettor.
f. Seal the PK Plate with the MicroAmp® Clear Adhesive Film and shake for 5 minutes at speed 8.
g. Add 240 µL of isopropanol to each sample, and proceed immediately to DNA isolation (next step).

4 Wash the beads and elute the DNA

a. Select the program on the instrument.
   - **4413021 DW blood** on MagMAX™ Express-96 Magnetic Particle Processor
   - **A25597_Blood_Buccal** on KingFisher™ Flex Magnetic Particle Processor
b. Start the run and load the prepared processing plates to their positions when prompted by the instrument (see Table 1).
c. Load the PK Plate (containing lysate, isopropanol, and Bead/RNase Mix) at position 1 when prompted by the instrument .
d. When prompted by the instrument (approximately 28–30 minutes into the run):
   1. Remove the Elution Plate from the instrument.
   2. Add 50 µL of DNA Elution Buffer 2 to each sample well.

   IMPORTANT! Add DNA Elution Buffer 2 immediately after the prompt to prevent excessive drying of any beads that are still captured on the Tip Comb.

   3. Load the Elution Plate back onto the instrument, and press **Start**.
e. At the end of the run (approximately 30–35 minutes after initial start), remove the Elution Plate from the instrument and seal immediately with a new MicroAmp® Clear Adhesive Film.
   - If precipitated DNA is visible, pipet up and down 5–10 times before sealing the plate, to ensure complete resuspension.

4	Wash the beads and elute the DNA *(continued)*	• Eluates can also be transferred to a new elution plate after collection. • If excess bead residue is seen in the wells, place the Elution Plate on the Magnetic Stand-96 to collect any residue prior to downstream use of the DNA. **IMPORTANT!** Do not allow the purified samples to sit uncovered at room temperature for more than 10 minutes, to prevent evaporation and contamination. The purified samples are ready for immediate use. Alternatively, store the covered Elution Plate: • At 2–6°C for up to 24 hours. • At –20°C or –80°C for long-term storage.

Recommended quantitation methods

Standard curve analysis is the most accurate quantitation method whereas UV absorbance measurements can be used to assess both the concentration and the quality of the isolated DNA.

- **Standard curve analysis.** Use the TaqMan® RNase P Copy Number Reference Assay (Cat. no. 4403326) for human genomic DNA and the TaqMan® DNA Template Reagents (Cat. no. 401970) to create a standard curve. Refer to *Creating Standard Curves with Genomic DNA or Plasmid DNA Templates for Use in Quantitative PCR* (Pub. no. 4371090).
- **UV absorbance measurements.** Use a NanoDrop® or other comparable instrument. Pure genomic DNA should have an A_{260}/A_{280} ratio of approximately 1.6–2.0.

Note: Mix the samples by pipetting up and down before quantitation if they have been stored frozen.

Revision history

Revision	Date	Description
C.0	July 2014	Addition of important procedural guidelines
B.0	July 2014	Correction of the kit name
A.0	May 2014	New document

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

B

Sample quantification using the RNase P Detection Reagents Kit

Overview

The Ribonuclease P RNA component H1 gene (RPPH1) is a single-copy gene encoding the RNA moiety (H1RNA) of the RNase P enzyme. It is a useful gene for quantifying amplifiable genomic DNA when used in conjunction with a standard curve generated from a genomic DNA standard of known concentration. The TaqMan® RNase P Detection Reagents Kit contains a 20X mix of primers and probe (FAM™ dye-labeled) that will amplify genomic copies of the RNase P RPPH1 gene.

Procedure

Materials required

Item	Catalog no.
TaqMan® RNase P Detection Reagents Kit	4316831
TaqMan® DNA Template Reagents Kit	401970
TaqMan® Genotyping Master Mix (2X)	4371355
(Optional) TaqMan® Fast Advanced Master Mix (for samples with PCR inhibitors)	4444557
Nuclease-free Water†	10977-015
MicroAmp® Optical Adhesive Film	4360954
MicroAmp® Optical Reaction Plate‡ • MicroAmp® EnduraPlate™ Optical 384-Well Clear Reaction Plates with Barcode • MicroAmp® Optical 96-Well Reaction Plate • MicroAmp® Fast Optical 96-Well Reaction Plate, 0.1 mL	 • 4483273 • 4316813 • 4346907

† Also provided in the MagMAX™ DNA Multi-Sample Ultra Kit
‡ Select one to fit your PCR instrument block

Prepare reagents, standards, and samples

1. Remove the 20X RNase P Primer-Probe mix from the TaqMan® RNase P Detection Reagents Kit and thaw at room temperature.
2. Remove the tubes labeled DNA Templates 1, 2, 3, 4, and 5 from the TaqMan® DNA Template Reagents Kit and thaw at room temperature.
3. Remove samples from storage and thaw at room temperature if necessary.
4. Vortex the reagents and samples from Steps 1–3 briefly, and centrifuge to collect contents at the bottom of the tubes.
5. Remove the TaqMan® Genotyping Master Mix from storage and thaw. Once thawed, mix gently by inversion.

Prepare reaction mix

1. Calculate the number of reactions based on the number of samples, standards, Negative Template Control (NTC), and replicates. This can be calculated using the following formula:

   Number of reactions =

   (5 standards + 1 NTC + no. of samples) × no. of replicates × 1.10 (overage factor for pipetting error)

   Example calculation for 36 samples and 4 replicates:

   Number of reactions = (5 + 1 + 36) × 4 × 1.1 = 185 reactions

2. Prepare the reaction mix by combining reaction components, except for genomic DNA, in an appropriately sized tube using the following table as example:

Component	Volume/reaction	Volume for 185 reactions (example)
2X TaqMan® Genotyping Master Mix	5 µL	925 µL
20X RNase P Primer-Probe mix	0.5 µL	92.5 µL
Nuclease-free Water	2.5 µL	462.5 µL
Total	**8.0 µL**	**1480 µL**

3. Vortex the tube gently and set aside.

Prepare the PCR reaction plate

1. Prepare the plate layout based on the number of samples and replicates. See the example layout below for 36 test samples with 4 replicates on a 384-well format PCR plate:

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	1	1	1	1	2	2	2	2	3	3	3	3	4	4	4	4	5	5	5	5	6	6	6	6
B	7	7	7	7	8	8	8	8	9	9	9	9	10	10	10	10	11	11	11	11	12	12	12	12
C	13	13	13	13	14	14	14	14	15	15	15	15	16	16	16	16	17	17	17	17	18	18	18	18
D	19	19	19	19	20	20	20	20	21	21	21	21	22	22	22	22	23	23	23	23	24	24	24	24
E	25	25	25	25	26	26	26	26	27	27	27	27	28	28	28	28	29	29	29	29	30	30	30	30
F	31	31	31	31	32	32	32	32	33	33	33	33	34	34	34	34	35	35	35	35	36	36	36	36
G	Std1	Std1	Std1	Std1	Std2	Std2	Std2	Std2	Std3	Std3	Std3	Std3	Std4	Std4	Std4	Std4	Std5	Std5	Std5	Std5	NTC	NTC	NTC	NTC
H																								
I																								
J																								
K																								
L																								
M																								
N																								
O																								
P																								

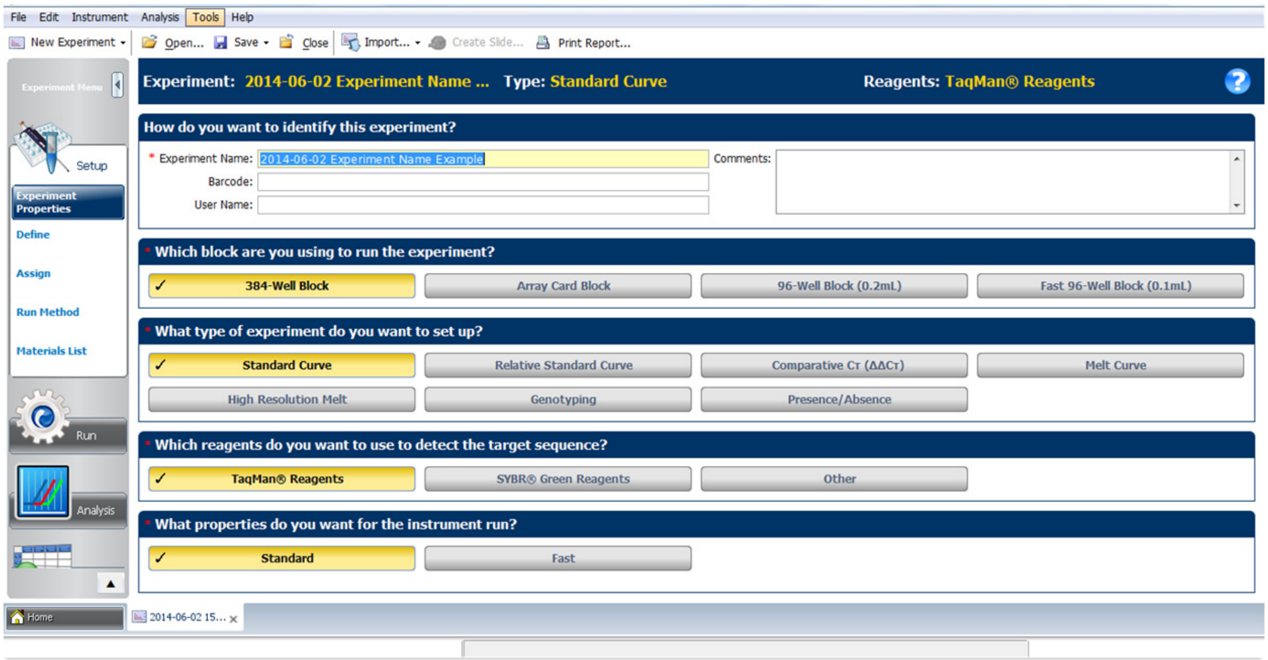
2. Pipet 8 µL of the reaction mix into the highlighted wells of the reaction plate.
3. Carefully aliquot 2 µL of the standards (DNA Templates 1, 2, 3, 4, and 5), unknown samples, and NTC (Nuclease-free Water) into the appropriate wells.
4. Seal the plate with a MicroAmp® Optical Adhesive Film, vortex briefly, and centrifuge in a plate centrifuge to collect the reaction at the bottom of the wells.

Setup and run the Standard Curve Experiment

1. Start the QuantStudio® System software.
2. Open a **New Experiment** and enter the name of the experiment (see figure below).
3. Select the appropriate block format (*384*, *Array Card*, *96*, or *Fast 96*).

4. Select *Standard Curve, TaqMan Reagents*, and *Standard* as highlighted below (when using TaqMan® Genotyping Master Mix).

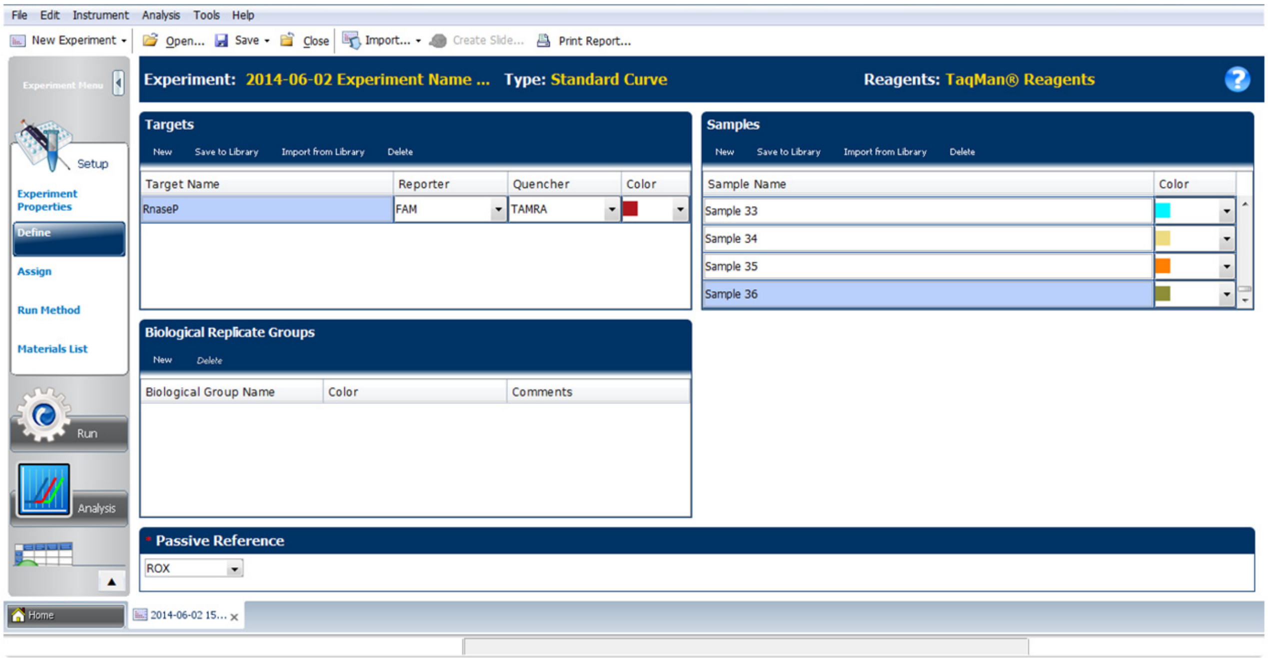
   IMPORTANT! If the TaqMan® Fast Advanced Master Mix is used, select the *Fast* run mode option.



5. Click the **Define** tab from the left window pane (see figure below.)

6. In the **Targets** pane, enter *RnaseP* for the Target Name, select *FAM* for Reporter, and *TAMRA* for Quencher.

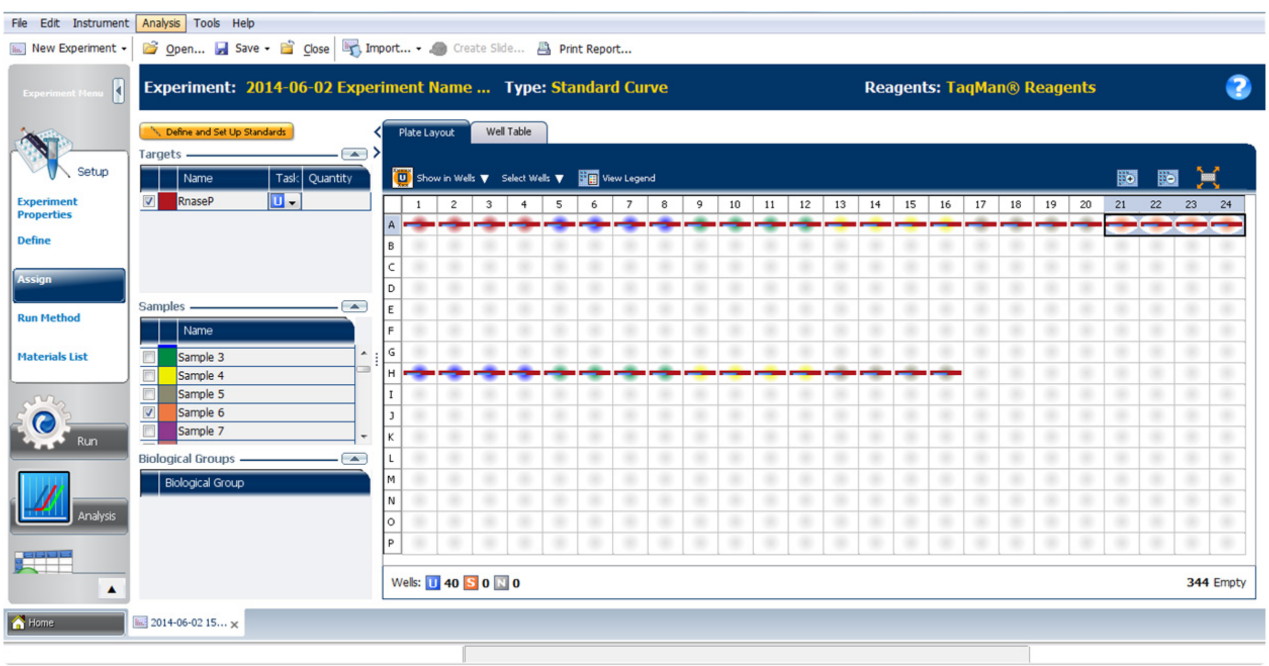
7. Sample names and standards can be entered in the **Samples** pane on the right side of the window.



8. Click the **Assign** tab on the left window pane to assign the samples and standards to well positions (see figure below).

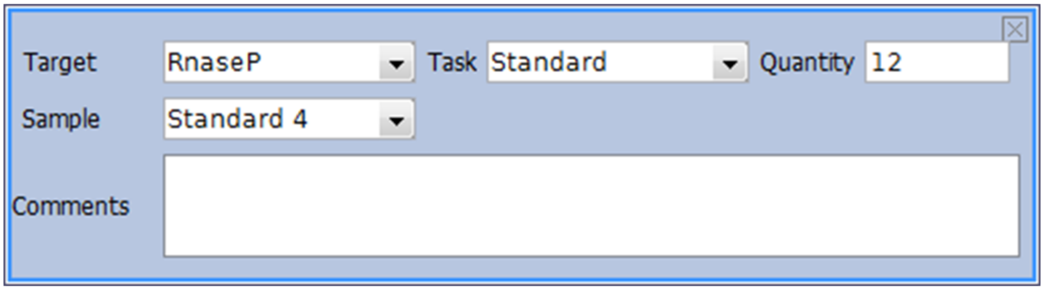
9. Select the well for each sample or standard and check the sample name from the **Sample** pane on the left.

10. Be sure to assign the *RnaseP* target for all the wells from the **Targets** pane.



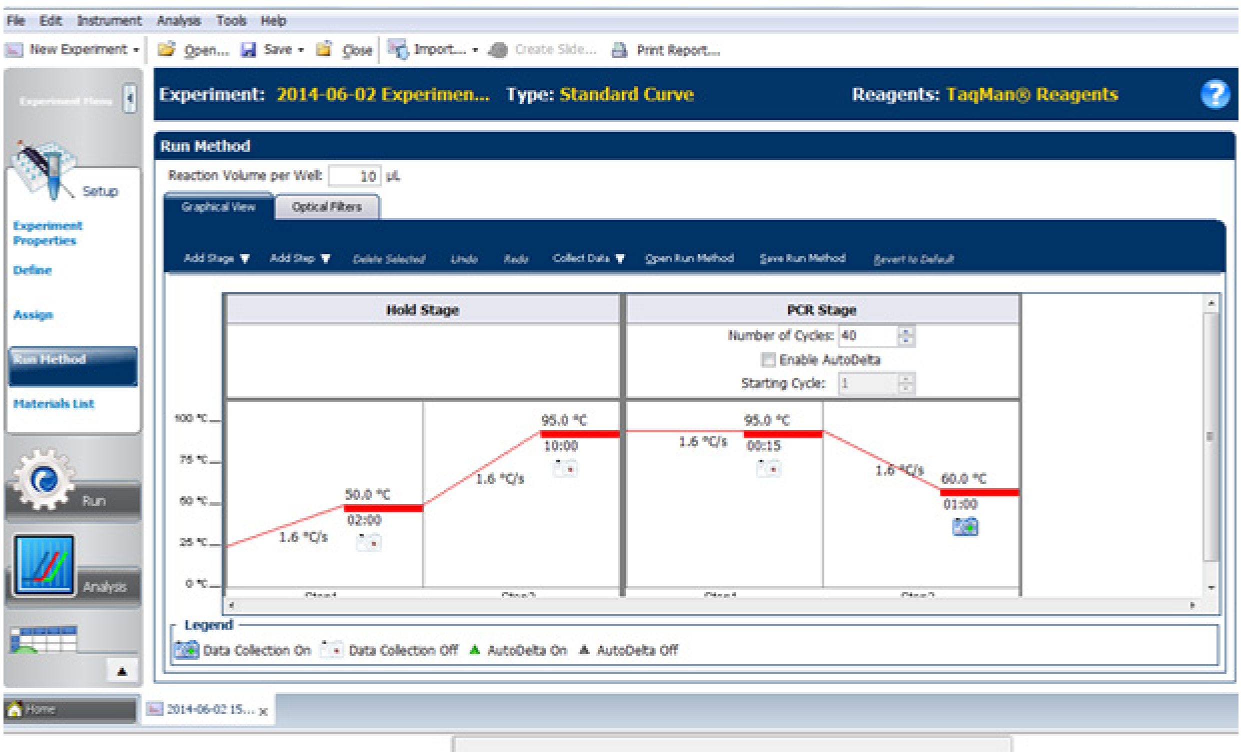
11. Enter the concentration for each standard by double-clicking on the well to activate a pop-up window.

12. Select *Standard* under **Task** and enter the concentration (**Quantity**) for the selected standard.



13. Once all of the samples and standard have been defined, click on the **Run Method** tab on left window to view the thermal cycling protocol.

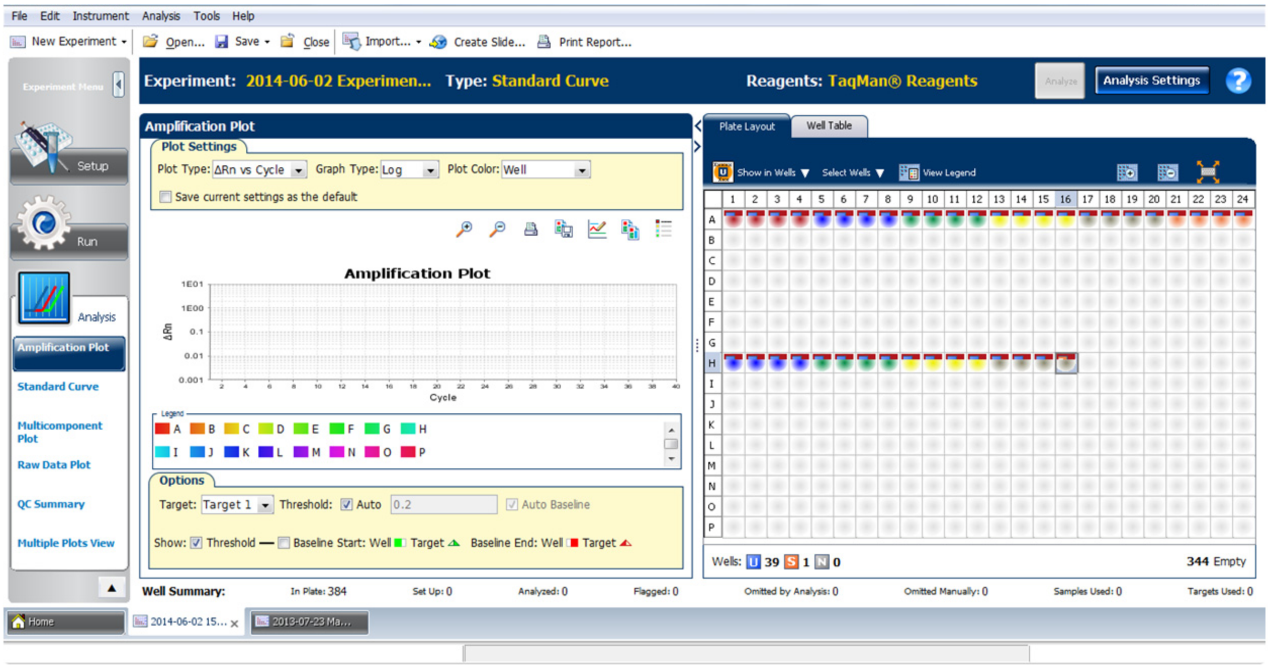
14. Change the reaction volume to 10 µL and confirm that the run protocol is identical to what is shown in the figure below.



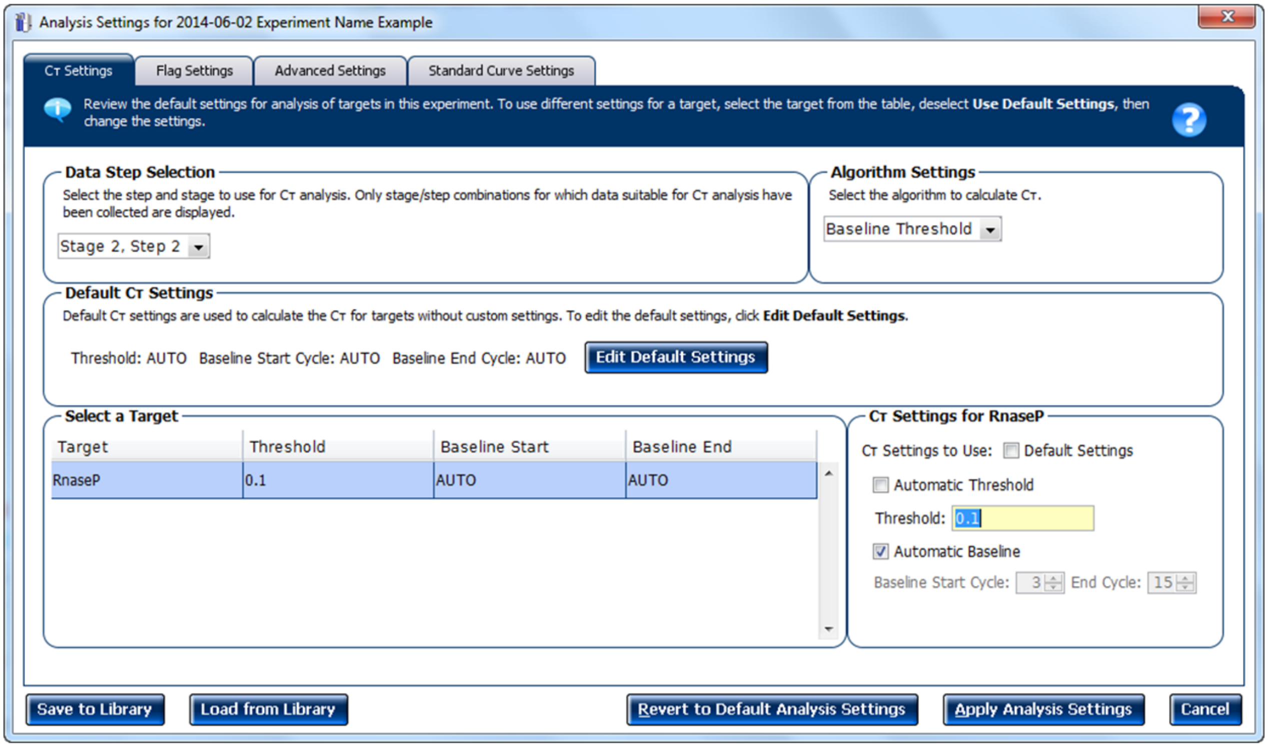
15. Select the **Run** tab on the left pane and start the run by clicking the green **Start Run** button.

Analyze and Export the Results

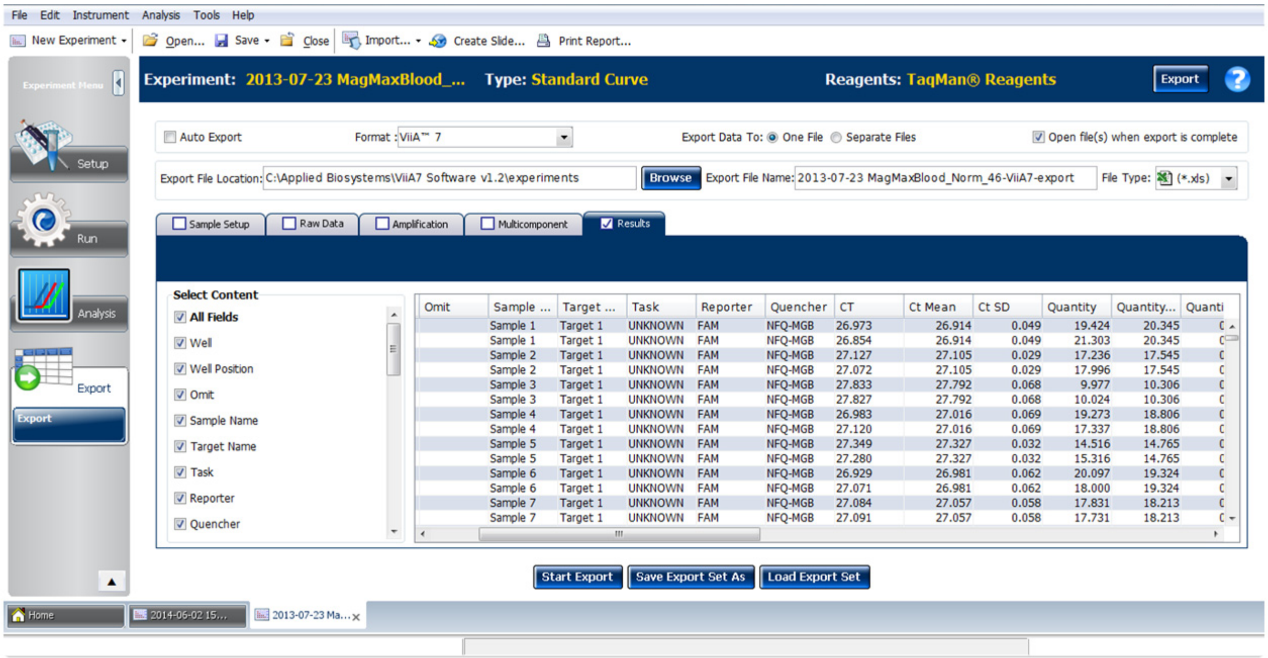
1. After the run is complete, select the **Analysis tab** from the left pane and then click the blue **Analysis Settings** button in the upper right corner to activate the **Analysis Settings** window.



2. In the **C_T Settings** tab, uncheck both the Default Settings and Automatic Threshold boxes that are in the lower right portion of the window. Set the **Threshold** value to 0.1. Be sure that the **Auto Baseline** box is checked. Then click **Apply Analysis Settings**.



3. Export the results by first clicking the **Export** tab in the left pane. **Browse** to destination file location for the export file and select the **Results** tab. Click on the blue **Start Export** button at the bottom of the screen to save the results file to the destination folder. The results file will have the quantification results for each sample in the **Quantity Mean** column of the spreadsheet.



4. Refer to your instrument's software user guide for further information.

C Troubleshooting

This appendix provides troubleshooting information for running PGx experiments using OpenArray® plates on the QuantStudio™ 12K Flex System.

Image analysis

Many problems with OpenArray® results can be diagnosed by examining the quality control (QC) images taken at various points during a cycling/imaging run. In the QuantStudio Software, with the experiment in question open, click **Export QC Images** in the Export tab. A set of images will be exported to the chosen folder location. Export a set of images to a unique folder, as exporting another run to the same folder will overwrite the images. The images exported are either fluorescent or reflected light images taken before, during and after cycling.

Image name	Description
BARCODE IMAGE.tiff	Reflected light image of the entire OpenArray® plate, useful for confirming the identity of a folder full of images.
PRE-READ_CHANNEL_4.tiff POST-READ_CHANNEL_4.tiff	Pre and post ROX images, useful for assessing the loading quality.
s00_c001_t01_p0001_m2_x3_e1_cp#_spotfind.tiff	Pre-run reflected light spotfinding image (used by the software for determining the location of the holes), useful for checking for existing contamination on the case and/or heated cover.
s03_c001_t03_p0001_m2_x3_e1_cp#_spotfind.tiff	Mid-run reflected light spotfinding image, useful for identifying potential leaks or other contamination that only appears mid-run.
s04_c001_t02_p0001_m2_x3_e1_cp#_spotfind.tiff	Post-run reflected light spotfinding image, useful for identifying potential leaks or other contamination.

Image name	Description
STAGEx_CYCLEy_CHANNEL_z.tiff	FAM (z=1) or VIC (z=2) images at a particular cycle (y) of a particular stage (x) of the run, useful for looking at patterns in the florescent data (e.g. gradients).

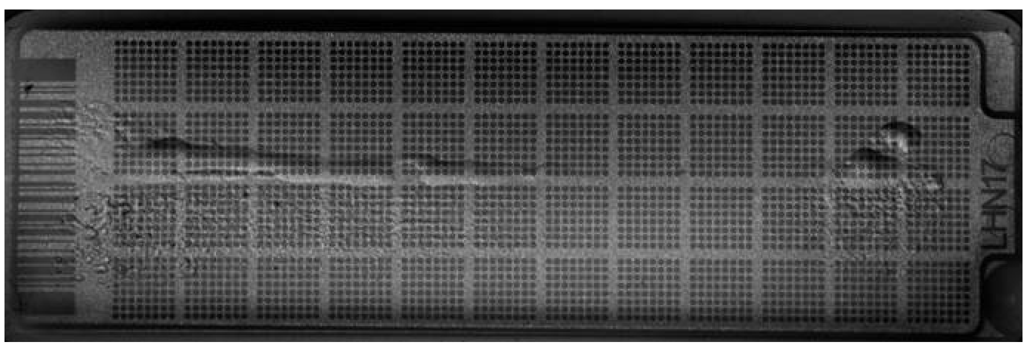
Note: "cp#" in the image file name refers to the position (1–4) within the QuantStudio™ 12K Flex instrument.

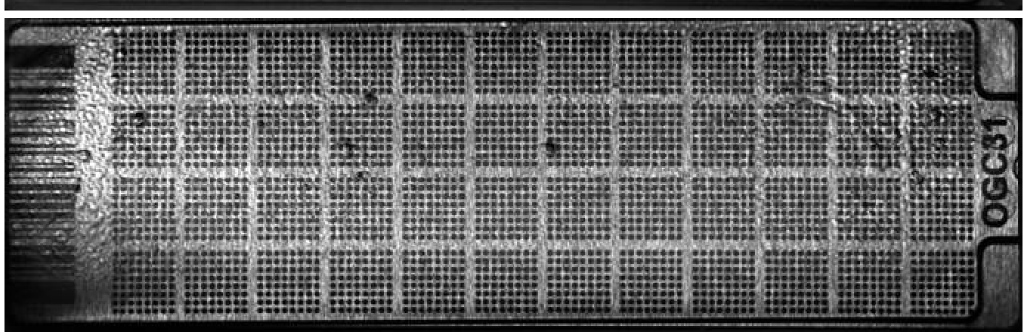
To view images, a free imaging program called ImageJ is recommended due to its ability to easily manipulate the images in ways that programs such as the built-in Windows image viewers can't. Many images will need to be adjusted for brightness/contrast in order to really be able to see what is happening. To do this, open the image in ImageJ, then open the brightness/contrast adjustment via **Image ▸ Adjust ▸ Brightness/Contrast** (or press **Ctrl+Shift+C**). Then, click the **Auto** button (or adjust the sliders) until the features of interest in the image are visible.

Case leaks and bubbles

Improper sealing of the OpenArray® plate in the OpenArray® Case can lead to immersion fluid leaks or bubble formation inside the case, leading to uneven heating and imaging throughout PCR and to poor quality genotyping data.

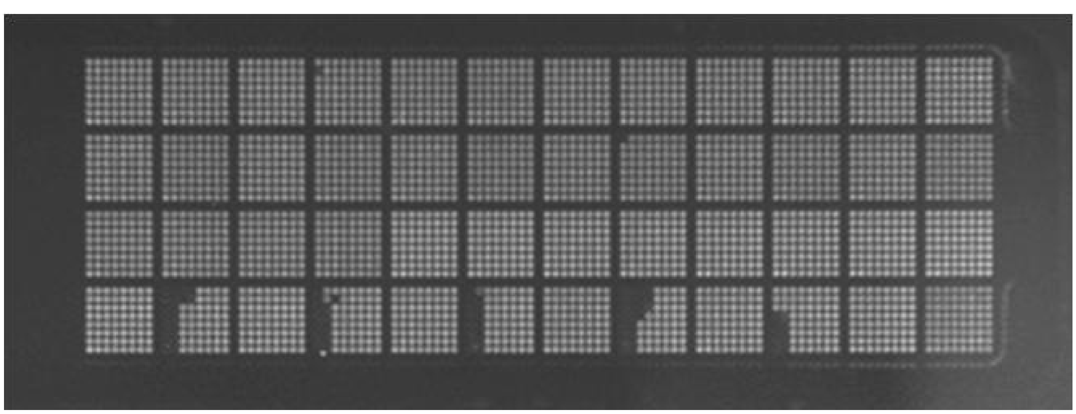
Below are some examples of OpenArray® plates that have been affected by immersion fluid leaks. The images show where leaked fluid has condensed on the underside of the heated cover windows and obscured the view of the through-holes.





Possible cause	Recommendation
Plate press was not engaged for at least 20 seconds	Ensure that the plate press is fully engaged for at least 20 second on future experiments.
Damaged lid adhesive	Visually inspect the lid adhesives for defect when the liner has been removed. Ensure that adhesive is not damaged or warped.
Damaged fill port screw gasket	Visually inspect the screw to ensure that the orange gasket is present and not damaged.
Damaged fill port screw assembly. Breaks off too easily	The screw may be mis-threaded: Unscrew it and use a new screw assembly.
Oily lid or case from immersion fluid overflow	Wipe off excess overflow of immersion fluid from the lid, case bottom, and crevices with 70% isopropyl alcohol, using a lint-free cloth (the cloth included with the OpenArray® plate is acceptable).
Immersion fluid exposed to air for too long	Do not remove the immersion fluid syringe cap or draw air bubbles into the syringe until you are ready to load. Do not draw air bubbles into the syringe.
Too large of a bubble inside the OpenArray® case after sealing	Minimize the size of the bubble by tilting the OpenArray® case so that the fill port is at the highest point. Slowly fill the case with immersion fluid until only a small air bubble remains. Attach the screw and wipe off any excess oil that may have spilled onto the case.
Damaged plate press leading to uneven pressure	Contact your field service engineer if you suspect that your plate press may be damaged.

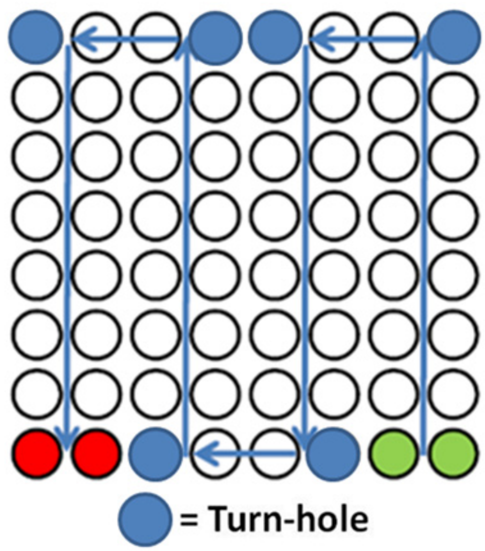
Sample plate preparation errors

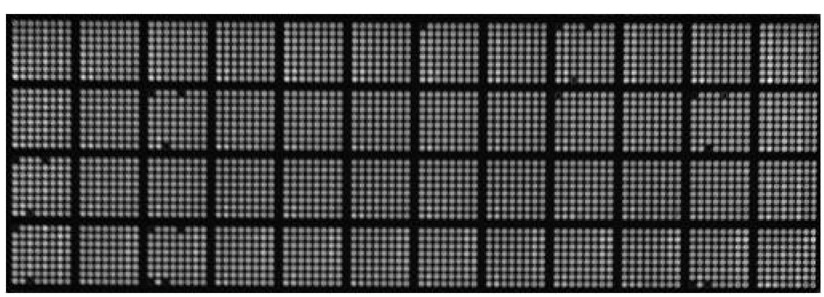


Possible cause	Recommendation
Not enough volume of sample was added to the 384-well sample plate	Ensure that proper pipettes techniques are performed. No air bubbles in the pipette tips after sample aspiration.
Reaction mix (sample + master mix) is not at the bottom of the 384-well sample plate	Ensure that the sample plate is centrifuged at 1000 rpm for 60 seconds.

AccuFill™ loading errors

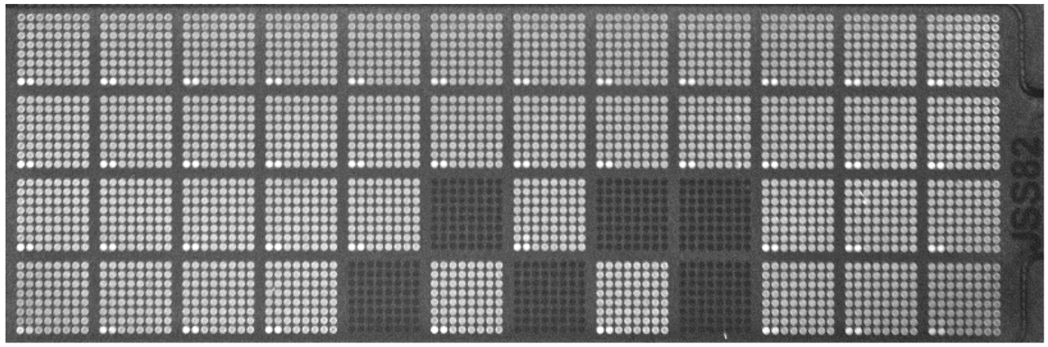
Systematic loading problems can occur with the AccuFill™ instrument, indicating a need for service. For example, when turnholes (where the AccuFill™ instrument changes direction during sample loading; see Load Path image below) are repeatedly missed across multiple subarrays, service is required.





Possible cause	Recommendation
AccuFill™ instrument is aligned too far to the left or right	Please contact your field service engineer to resolve the issue.

Entire subarrays missing

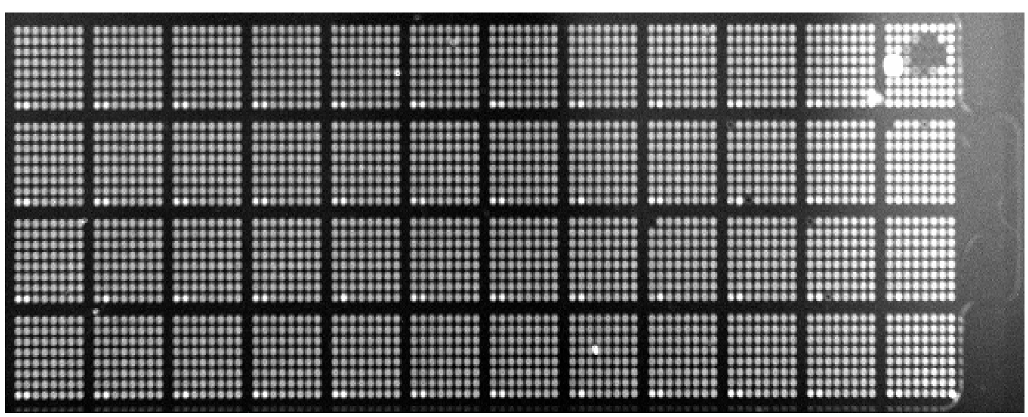


Possible cause	Recommendation
Sample/Master Mix not added to particular wells in the 384-well sample plate	Visually inspect the sample plate to confirm that the wells have sample/ master mix.
Stuck tip mandrel on AccuFill™ instrument may need cleaning	Contact your local field service engineer.
Pipette tip not loaded on mandrel	Contact your local field service engineer if this happens regularly (infrequent occurrences can be due to a poorly molded tip).

OpenArray® plate assembly and handling errors

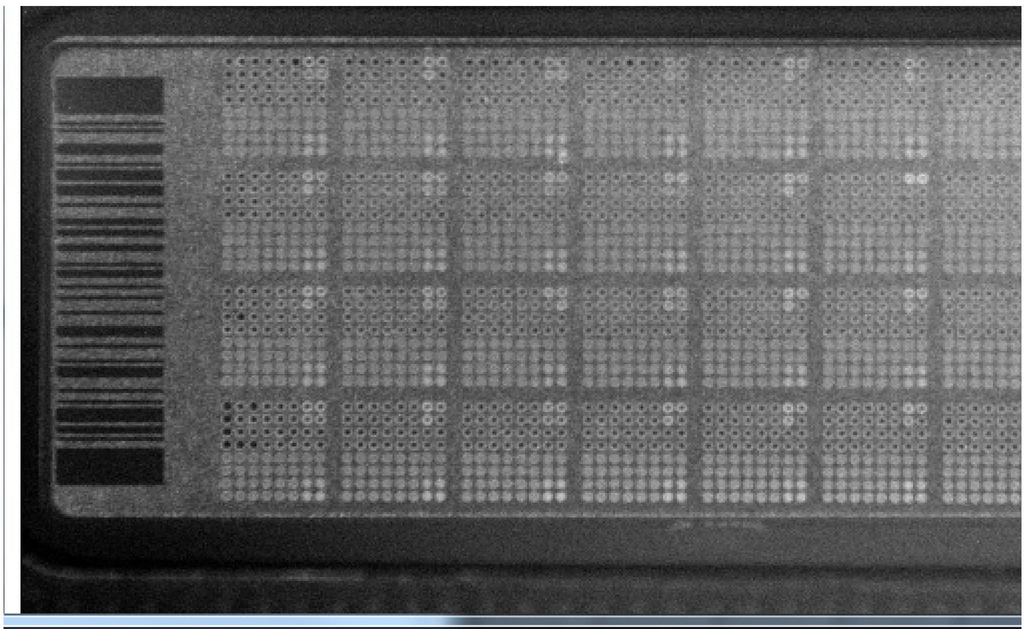
Refer to the *QuantStudio™ 12K Flex Real-Time PCR System OpenArray® Experiments User Guide* (Pub. no. 4470935) for detailed troubleshooting information.

Sample blow out during the addition of immersion fluid



Possible cause	Recommendation
The reactions in A12 were compromised during the addition of immersion fluid. Injecting the immersion fluid too quickly can actually purge the sample out of the through holes near the fill port. Often this is caused by the user not purging the syringe slightly before use.	A small amount of immersion fluid should be dispensed onto a paper towel before use to ensure smooth operation of the syringe.

Evaporation of reaction mixture



Possible cause	Recommendation
Too much time elapsed before plate was sealed with lid and immersion fluid. In this example, the top half of each subarray was intentional left open to the environment to demonstrate the effect of evaporation. "Donuts" are a result of the evaporated fluid in the though-holes	To minimize the likelihood of evaporation, take the plate off of the AccuFill™ deck, seal the case with the lid, and add immersion fluid as soon as the case is removed from the plate press.

Stability of assembled OpenArray® plates

Sealed OpenArray® plates should be run within one hour of assembly. If necessary, genotyping plates may be stored at 4°C to 8°C or at room temperature (do not freeze) for up to 24 hours before cycling. However, immediate cycling is recommended for best results. If plates have been stored at 4°C to 8°C, allow them to equilibrate to room temperature before cycling. If an OpenArray® plate is not cycled immediately after loading, minimize light exposure to prevent photobleaching of the dyes.

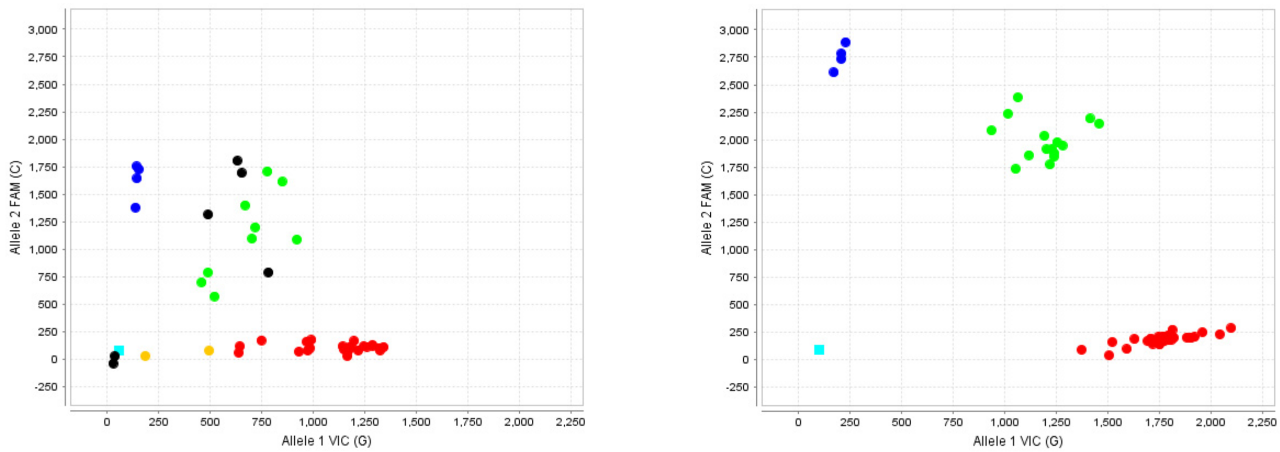
Insufficient sample input

Diffused clusters and loss of heterozygosity may be due to an insufficient amount of starting DNA concentration (50 ng/µL recommended).

In addition, there may be an abundance of samples that did not amplify (ones that are called as "noamp" and that run close to NTCs in genotyping plots).

If it is not possible to increase the concentration of the starting material, preamplification is recommended. See the preamplification protocol in Chapter 4 "(Optional) Preamplify DNA samples" on page 36.

The image below shows poor quality SNP data (left) that is improved by preamplifying the samples (right):

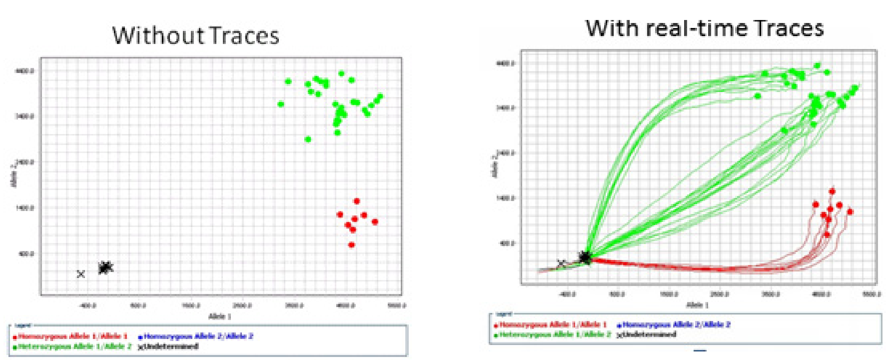


Using QuantStudio™ 12K Flex Software to troubleshoot and make genotyping calls

See "Analysis settings in the QuantStudio® 12K Flex Software" in Chapter 5 on page 39 to view real-time traces in the QuantStudio™ 12K Flex Software.

Real-times traces can be used to identify outlier data points and make manual calls

In the example below, looking at data from an earlier cycle number helped in determining the correct genotypes for the samples in question. On the left, the QuantStudio™ 12K Flex Software called many data points heterozygous. However, the real-time traces show signal saturation of the homozygous cluster towards the heterozygous cluster. As a result, we have good evidence to change some of the calls from heterozygous to homozygous for the FAM™ allele.



The QuantStudio™ 12K Flex Software can be used to view amplification curves. As a rule of thumb, a C_T-value of about 25 indicates that the sample input is approximately 250 haploid copies, which is the recommended input amount per 33-nL reaction for OpenArray® genotyping plates (i.e., 825 pg of DNA will be loaded per PCR when a stock solution of 50 ng/µL is used).

Expected versus unexpected no amps and undetermined calls

For certain assays, it is expected that there will be some samples that legitimately fail to amplify or to get a genotype call. These include samples run with assays that interrogate gene variants that are associated with copy number variation and with assays that detect triallelic SNPs or adjacent SNPs. See “Assays that require manual genotype calls” in Chapter 5 on page 39 for details on how to analyze the data for such assays.

TaqMan® DME genotyping assays to genes in copy number variation regions

As detailed in Chapter 5, some DME Assays target polymorphisms in genes that exhibit Copy Number Variation. Copy number variation analysis must be done in addition to genotyping with DME assays.

- If both copies of a gene are deleted in a sample (copy number of 0), samples will not be amplified and will run near or with the NTCs.
- If a sample carries more than 2 copies of a gene and both SNP alleles are present, it will fall within the heterozygous cluster or occasionally to one side or the other of it, in which case it may be called as undetermined. These should be manually called as heterozygous for data analysis purposes.

TaqMan® DME genotyping assays to triallelic SNPs and adjacent SNP targets

As detailed in Chapter 5, “Prepare, run, and analyze OpenArray® PGx experiments”, triallelic SNP and adjacent SNP targets can be interrogated using a pair of TaqMan® SNP assays. Each assay contains one probe for the major SNP allele or haplotype, and one probe for one of the minor alleles or haplotypes. After running paired assays in separate reactions on the same genomic DNA samples, the results of the 2 assays are compared to determine the sample genotype. For a given assay it is expected that:

- Samples having just one reported allele may run close to or within a homozygous cluster. Any samples running close to homozygous clusters that are called as undetermined should be manually called as homozygous for data analysis purposes.
- Samples that are homozygous for the unreported allele may cluster with NTCs or may exhibit weak amplification due to probe nonspecific activity. If a weakly amplifying sample is called as undetermined, manually adjust the call to “noamp”.

D Expected performance and system specifications

The following tables provide system specifications. Refer to the *OpenArray® technology on the QuantStudio® 12K Flex System Product Bulletin* (Pub. no. CO23802) for more detail on performance and specifications.

Table 12 QuantStudio® 12K Flex OpenArray® AccuFill™ System specification

Description	Specification
Fill rate	99.25%
Maximum allowable sample carryover	<1%
Maximum allowable assay carryover	<1%
Maximum number of through hole data points lost due to evaporation	<5%

Table 13 QuantStudio® 12K Flex TaqMan® OpenArray® Genotyping Plates

Description	Specification
Assay conversion rate from a 7900HT Real-Time PCR system	>95%†
Concordance with assays run on a 7900HT Real-Time PCR system	99.7%†
Call rate	95%†
Number of haploid copies of gDNA/through-hole	250
TaqMan® OpenArray® Genotyping Plate capacity per 8 hr day by a single person	12
Time from purified DNA to genotyping data	~5 hr
Throughput of one technician in one day	110,000‡

† Individual performance is dependent on the integrity and purity of samples.

E

Genotyping and copy number controls

This appendix covers:

Overview

Many laboratories desire to test the performance of their selected TaqMan® SNP and DME Genotyping assays on OpenArray™ plates by using samples that represent all three genotypes (homozygous major allele, homozygous minor allele, and heterozygous) to test for both amplification and cluster separation. Some laboratories prefer to use samples that represent at least two genotypes (homozygous major and minor alleles, or homozygous major allele and heterozygous) to test that both assay probes function. Ideally, gDNA samples of known genotypes can be used for such experiments. However, for rare DME variants that are not well-represented in populations, it may be necessary to use synthetic templates.

This Appendix includes information on sources of control cell line sample DNAs for DME and SNP genotyping assays, as well as for copy number assays. It also includes information on ordering synthetic template DNAs in plasmids.

Cell line gDNA controls

This section describes some publicly available sources for reference or control materials that can be used in genetic testing and assay performance experiments.

TaqMan® Drug Metabolism Genotyping Assays test data

All TaqMan® DME Genotyping Assays were run on 180 unique DNA samples from four different populations in assay performance and reproducibility testing. Samples tested included a panel of 45 African American and 45 Caucasian samples obtained from the Coriell Cell Repositories and a panel of 45 Japanese and 45 Chinese samples provided by a collaborator. The minor allele frequencies determined for each assay are provided on the Life Technologies web site (assay search results) and in the DME Index. It is important to note that many of the polymorphisms interrogated by this assay collection have very low minor allele frequencies (MAFs) of less than 1%; thus the minor allele was not detected for all assays within the 180 test samples.

For TaqMan® DME assays having a reported Applied Biosystems® (AB) MAF in the African American or Caucasian populations, heterozygous or minor allele homozygous samples may be used as reference or control samples in genetic testing and assay performance studies. A complete list of the samples used and their genotypes is available at **www.lifetechnologies.com**.

Order the gDNA samples from the Coriell Cell Repositories at **http://ccr.coriell.org**. Please note that the genotypes of these samples were, for the most part, not verified by sequence analysis.

NCBI dbSNP genotype data

Another source of DME assay control samples is the rsSNP submissions in the NCBI database of Short Genetic Variations (dbSNP) at **http://www.ncbi.nlm.nih.gov/SNP**. One can search by rsSNP to navigate to specific rsSNP pages that may have genotype information listed in the Population Diversity table. Submissions from sources such as the HapMap project used Coriell samples; the sample genotypes may be found by clicking on the submission ss # to navigate to the submission details page. For HapMap samples, the sample genotypes may also be found at the HapMap web site **http://hapmap.ncbi.nlm.nih.gov**.

Centers for Disease Control and Prevention (CDC) reference materials

Clinical Laboratory Improvement Amendments (CLIA)

http://wwwn.cdc.gov/clia/Resources/GETRM/default.aspx

The Centers for Disease Control and Prevention (CDC) provides genetic information on cell line DNAs that can be used as reference materials for genetic testing and assay validation. Some of these cell lines were characterized by the Genetic Testing Reference Materials Coordination Program (GeT-RM). One major focus category is the 'Genetic Inherited Disease & Pharmacogenetics' section. Tables of reference samples that contain PGx/DME or disease allele variants, many of which have been confirmed by multiple labs and genetic testing technologies, can be downloaded from this site:

http://wwwn.cdc.gov/clia/Resources/GETRM/MaterialsAvailability.aspx

TaqMan® Copy Number Assays for DME genes

Several DME genes occur in CNV regions (see "Special assay considerations" in Chapter 2, "Background and tools for assay content selection" on page 13). In addition, the CYP2D6 and CYP2A6 genes are known to recombine with related pseudogene sequences to generate hybrid genes, many of which have decreased or null gene activity. TaqMan® Copy Number Assays can be used to detect deletions, duplications, and hybrid gene alleles. TaqMan® Copy Number Assays for the major known DME genes that exhibit CNV have been pre-tested on a panel of 45 each African American and Caucasian (the same samples as were used for TaqMan® DME Genotyping Assay validation studies). A complete list of the samples used and their genotypes is available at **www.lifetechnologies.com/pgx**.

The gDNA samples can be ordered from the Coriell Cell Repositories (**http://ccr.coriell.org**).

Plasmid controls

Life Technologies has successfully used synthetic major and minor allele template DNAs cloned into plasmids to test the performance of over 300 TaqMan® DME assays. In addition to major or minor allele sequences, plasmids carry the RNase P RPPH1 gene for accurate quantitation of plasmids by the standard curve analysis before use in genotyping experiments. Equal quantities of major and minor allele plasmids are mixed together to create heterozygous controls. The homozygous and heterozygous plasmid controls can be used to demonstrate amplification and detection of all three

genotypes by a given assay. Plasmid control samples will often, but not always, cluster with gDNA samples. Example data can be found in the TaqMan® DME Genotyping Assays on OpenArray® Plates .ppt file that can be downloaded from: **www.lifetechnologies.com/pgx**

For information on ordering TaqMan™ DME and SNP genotyping assay plasmid controls, please send an email to: **QuantStudioFrontDesk@lifetech.com**.

F

Good PCR practice

Prevent contamination and nonspecific amplification

PCR assays require special laboratory practices to avoid false positive amplifications. The high throughput and repetition of these assays can lead to amplification of one DNA molecule.

PCR good laboratory practices

When preparing samples for PCR amplification:

- Use a positive-displacement pipette or aerosol-resistant pipette tips.
- Follow proper pipette-dispensing techniques to prevent aerosols.
- Wear clean gloves and a clean lab coat (not previously worn while handling amplified PCR products or used during sample preparation).
- Change gloves whenever you suspect that they are contaminated.
- Maintain separate areas and dedicated equipment and supplies for:
  - Sample preparation
  - PCR setup
  - PCR amplification
  - Analysis of PCR products
- Do not bring amplified PCR products into the PCR setup area.
- Open and close all sample tubes carefully. Centrifuge tubes before opening. Avoid splashing or spraying PCR samples.
- Keep reactions and components capped as much as possible.
- Clean lab benches and equipment periodically with 10% bleach solution. Use DNA*Zap*™ Solution (Pub. no. AM9890).

G Safety

This appendix covers:

General safety

WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, etc). To obtain SDSs, see the "Documentation and Support" section in this document.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below, and consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the “Documentation and Support” section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

Biological hazard safety

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective equipment, which includes but is not limited to: protective eyewear, face shield, clothing/lab coat, and gloves. All work should be conducted in properly equipped facilities using the appropriate safety equipment (for example, physical containment devices). Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially infectious materials. Read and follow the applicable guidelines and/or regulatory requirements in the following:

In the U.S.:

- U.S. Department of Health and Human Services guidelines published in Biosafety in Microbiological and Biomedical Laboratories found at: **www.cdc.gov/biosafety**
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030), found at: **www.access.gpo.gov/nara/cfr/waisidx_01/ 29cfr1910a_01.html**
- Your company's/institution's Biosafety Program protocols for working with/ handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: **www.cdc.gov**

In the EU:

Check local guidelines and legislation on biohazard and biosafety precaution and refer to the best practices published in the World Health Organization (WHO) Laboratory Biosafety Manual, third edition, found at: **www.who.int/ csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/**

Documentation and support

Related documentation

The following related documents provide details supporting the PGx workflow:

Document	Pub. no.	Description	Related PGx Chapter(s)
TaqMan® Drug Metabolism Genotyping Assays for Triallelic SNPs Application Note	135AP01-01	An annotated application note that provides details on manual analysis of triallelic SNPs using 2 DME SNP assays.	Chapter 2, Background and tools for assay content selection
AlleleTyper™ Software User Guide	4469874	Provides reference information for the use of AlleleTyper™ Software and describes how to prepare translation tables containing genetic pattern information and how to use these translation tables to map TaqMan® SNP Genotyping Assay and/or TaqMan® Copy Number Assay results to any desired nomenclature used by a laboratory or research group.	Chapter 2, Background and tools for assay content selection Chapter 7, Perform translation analysis in AlleleTyper™ Software
MagMAX™-96 DNA Multi-Sample Kit MagMAX™ Express-96 Magnetic Particle Processor Protocol	4428202	Sample preparation guidance and instruction on using the MagMAX™ Express-96 Magnetic Particle Processor.	Chapter 4, Prepare samples Chapter 6, Prepare, run, and analyze copy number experiments
Creating Standard Curves with Genomic DNA or Plasmid DNA Templates for Use in Quantitative PCR Application Note	4371090	An application note providing detailed instructions for quantification using standard curve analysis.	Chapter 4, Prepare samples
OpenArray® Sample Tracker Software Quick Reference Guide	4460657	Instructions for loading samples into a TaqMan® OpenArray® plate.	Chapter 5, Prepare, run, and analyze OpenArray® PGx experiments
Applied Biosystems® QuantStudio® 12K Flex Real-Time PCR System: OpenArray® Plate Quick Reference Guide	4478673	Instructions for calibrating the instrument and performing gene expression experiments.	Chapter 5, Prepare, run, and analyze OpenArray® PGx experiments
QuantStudio® 12K Flex Software Help	–	Detailed online Help for batch file setup in QuantStudio® 12K Flex Software.	Chapter 5, Prepare, run, and analyze OpenArray® PGx experiments

Document	Pub. no.	Description	Related PGx Chapter(s)
QuantStudio® 12K Flex Real-Time PCR System OpenArray® Experiments User Guide	4470935	Provides detailed information about genotyping experiments in *Booklet 2 - QuantStudio® 12K Flex OpenArray® Genotyping Starter Kit*.	Chapter 5, Prepare, run, and analyze OpenArray® PGx experiments
QuantStudio® 12K Flex Real-Time PCR System Maintenance and Administration User Guide	4470689	Maintenance procedures include information on networking and transferring files.	Chapter 5, Prepare, run, and analyze OpenArray® PGx experiments
TaqMan® Genotyper™ Software Getting Started Guide	4448637	Detailed description of TaqMan® Genotyper™ analysis.	Chapter 5, Prepare, run, and analyze OpenArray® PGx experiments
TaqMan® Copy Number Assays Protocol	4397425	Full instructions for running TaqMan® Copy Number Assay experiments	Chapter 6, Prepare, run, and analyze copy number experiments
CopyCaller® Software v2.0 User Guide	4400042	A complete description of CopyCaller® Software features and guidance on using the software	Chapter 6, Prepare, run, and analyze copy number experiments
OpenArray® technology on the QuantStudio® 12K Flex System Product Bulletin	CO23802	Descriptive and ordering information for QuantStudio® 12K Flex System.	Appendix D, Expected performance and system specifications

Note: For additional documentation, see "Obtaining support" on page 120.

Obtaining SDSs

Safety Data Sheets (SDSs) are available from **http://www.lifetechnologies.com/support**.

Note: For the SDSs of chemicals not distributed by Life Technologies, contact the chemical manufacturer.

Obtaining Certificates of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Obtaining support

For the latest services and support information for all locations, go to:

www.lifetechnologies.com/support

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com**)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

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